ANATOMICAL AND BEHAVIORAL CONSEQUENCES OF EARLY BRAIN DAMAGE IN THE RABBIT

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To My Wife, Kathy,

whose confidence in me

has often been a source of encouragement

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OF EARLY BRAIN DAMAGE IN THE RABBIT

By

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Chairman: Dr. Robert L. Isaacson
Major Department: Psychology

In an effort to establish a morphological basis for the recovery of function which often follows early brain lesions in mammals, 56 young rabbits were subjected to unilateral or bilateral aspiration lesions of the posterolateral neocortex overlying the hippocampus or to combined lesions of cortex and hippocampus. An additional 34 young rabbits served as unoperated control subjects. The lesions were induced at ages varying from 1 day (neonatal) to 11 weeks in different rabbits. The animals were sacrificed after varying postoperative intervals which ranged from immediately after surgery to 14 weeks postoperatively.

Small circular aspiration lesions 3 mm in diameter induced at 1 day of age produced a stunting of growth in the damaged hemisphere(s) of subjects with either unilateral or bilateral lesions. The relative difference in size be-

tween damaged and undamaged hemispheres increased through the first week of life. At the end of the first week of life, the volume of the damaged hemisphere(s) of neonatal damaged subjects was approximately 20% smaller than the volume of the undamaged hemisphere(s). This relative difference in hemisphere size remained roughly constant (i.e., 15-20%) thereafter. In unilateral damaged subjects, the size of the damaged hemisphere was comparable to the size of the hemispheres of bilateral damaged subjects of the same age; and the undamaged hemisphere was comparable in size to the hemispheres of normal subjects of the same age. This difference in overall hemisphere size was reflected in statistically significant decreases in thickness of cortex, white matter, hippocampus, and septum; and in decreased width of diencephalon and midbrain in the damaged hemisphere. These size differences between various structures in damaged and undamaged hemispheres extended over considerable distances from the lesion site, although in general the degrec of disparity was greatest at the level of the lesion and decreased progressively with increasing distance from the lesion site.

Lesions of the same absolute magnitude induced in 6 day old rabbits also produced a stunting of hemisphere growth but this stunting was not as severe as that produced by neonatal lesions. Identical lesions sustained at 6 weeks of age or later produced no significant size differences between damaged and undamaged hemispheres.

The absolute size of the lesions increased during the

first postoperative week, reached a maximum at some point between 1 and 3 weeks postoperatively, and decreased progressively with longer postoperative intervals. The results suggested that this initial increase and subsequent decrease in lesion size was primarily a function of postoperative interval and was not dependent on the age of the animal at the time of surgery.

Correlated with the reduced size of the damaged hemisphere in neonatal damaged subjects was an increase in cellular packing density for neurons and glia as well as a large increase in glia-neuron index in the damaged hemispheres (as compared with the intact hemispheres).

Despite these rather dramatic morphological changes produced by early brain lesions, there were no observed lesion effects on any of several measures of perceptual-motor development in the young rabbit.

Recent evidence for several types of morphological plasticity in the mammalian brain is discussed in terms of the possible role of these mechanisms in producing functional recovery after early brain lesions.

GENERAL INTRODUCTION

It is now a well known fact that animals sustaining damage to many brain areas in infancy show a much less severe functional deficit than animals sustaining similar damage later in life. In other words, the functional loss observed depends not only on the size and location of the brain lesion but also on the age of the animal when the lesion is induced.

More than 30 years ago, Kennard (1938) reported that lesions of the motor cortex in infant monkeys produced less motor debility than did lesions at later ages. In fact, no deficit was observed in monkeys sustaining lesions before 3 weeks of age, whereas lesions sustained after 7 months of age resulted in severe motor impairment which showed no improvement during the 48 day postoperative survival period (Kennard, 1942).

At about the same time, Beach (1938) demonstrated less impairment in rat maternal behavior after infant cortical lesions than after lesions produced in adults; and Tsang (1937a, b) reported analagous results for visual perception and maze learning after neonatal cortical lesions in the rat. More recent investigations have extended knowledge of this phenomenon of functional sparing following neonatal lesions to somatosensory cortex in cats (Benjamin and Thompson, 1959),

auditory cortex in cats (Sharlock, Tucker, and Strominger, 1963), visual cortex in kittens (Doty, 1964), frontal association cortex in monkeys (Akert, Orth, Harlow, and Schiltz, 1960; Harlow, Blomquist, Thompson, Schiltz, and Harlow, 1968; Kling and Tucker, 1968; Goldman, Rosvold, and Mishkin, 1970), posterior association cortex in monkeys (Raisler and Harlow, 1965), and combined lesions of frontal and posterior association cortex in monkeys (Tucker and Kling, 1969). Finally, Isaacson, Nonneman, and Schmaltz (1968) have demonstrated that this phenomenon is not restricted to damage of the neocortex. They reported sparing of function after early lesions of hippocampus in cats, at least on some tasks.

One of the earliest attempts to account for sparing or recovery of function was the concept of vicarious function, the idea that one brain area takes over functions which it did not previously mediate. However, several facts make it likely that this commonly accepted notion, at least in its most literal form, is an inadequate generalization at best.

First, sparing of function occurs only on some tasks but not at all on others (Isaacson, et al., 1968; Goldman, et al.,1970). Furthermore, it often does not occur at all if the lesion is extensive enough or if it includes more than one structure in a system (Kennard, 1938; Kling and Tucker, 1968). Also, the degree of debility observed depends on the ontogenetic age of the subject at the time of testing as well as the age at which the brain damage occurred (Harlow, et al.,

1968). In some cases, the effects of early brain injury may lie dormant or hidden, occurring only after the subject reaches a certain stage of development (Lenneberg, 1968). Finally, from the work of Isaacson, et al. (1968), it appears that neonatal brain damage may never be totally without behavioral effect. Although their infant brain damaged subjects were unimpaired on many tasks in terms of trials or errors to criterion, the pattern of behavior of these subjects and the way in which they solved the problems was not like that of normal subjects.

In view of this recent evidence, it is clear that a simple substitution or vicarious function type notion must be replaced with a somewhat more complex interaction or functional reorganization notion. Specific brain structures probably never act in complete isolation. Instead, they interact, or function in consort with other functionally related structures in complex physiological systems. It would be naive to think of the effects of a lesion at any age as being restricted entirely to the area which has been damaged. Destruction of one area is bound to have physiological consequences on other structures throughout the system or systems with which it is related. Such partial destruction of a system is then considered to cause morphological and/or physiological reorganization of the rest of the system. This would be particularly likely in the case of infant lesions in which damage is imposed on a situation of rapid growth and development. It would be this function-

ally altered system which would then mediate the behavior of the animal, and as a result the behavior would almost certainly differ in some respect from that of a normal intact subject.

What then is the exact nature of the adjustive process which occurs after neonatal brain damage and which gives animals with early lesions an advantage over those with later lesions on certain types of problems? Also, how do the behavioral modifications observed in adulthood come to be the way they are? These are the questions with which this dissertation is concerned.

The first chapter presents a series of experiments on the effects of neonatal and early postnatal brain damage on the morphological development of the brain of the domestic rabbit, Orychtolagus cuniculus (L.). The second chapter presents a series of behavioral experiments on the effects of the observed morphological changes induced by early brain lesions on the development of perceptual-motor abilities in two races of domestic rabbits. Hopefully, a comparison of the observed morphological adjustments to early brain damage with their effects on early behavioral development may provide some insight into the mechanisms responsible for recovery of function following early brain lesions.

CHAPTER 1:
PROGRESSIVE MORPHOLOGICAL CHANGES AFTER ASPIRATION LESIONS OF THE BRAIN

Experiment 1: Effects of Neonatal Cortical Damage

Isaacson, et al. (1968), reported that the brains of many of their animals which had suffered neonatal destruction of either cortex or hippocampus showed very little gross evidence of the destruction when examined two or three years after the original surgery. In fact, the only evidence of the original insult in some animals was an altered gyral pattern. In other words, the original lesion seemed to have filled in with tissue during the 2-3 year interval between surgery and sacrifice. This filling of the lesion was not restricted to animals with neonatal destruction, but it appeared that the degree of filling was greater the earlier the lesion was induced. However, since the animals with early lesions usually survived for longer postoperative periods, it was not possible to say with certainty whether this effect was due to the age of the animal at the time of surgery or to the length of the post-operative interval.

For this reason, Nonneman and Isaacson (unpublished study) subjected newborn and 6 week old kittens to unilateral destruction of cortex or hippocampus. All of the subjects were sacrificed 6 months after surgery. Since the brains of both neonatal and 6 week damaged animals showed

large surface defects, it appears that, at least in cats, the filling-in process occurs over a long period of time. The most striking result of this study, and one which was not anticipated, was the fact that the damaged hemisphere was considerably smaller than the intact hemisphere, both in cortically damaged and in hippocampally damaged subjects. The overall size difference was of a magnitude (10-15% in Ss operated at 6 weeks of age; 20-25% in neonatal damaged Ss) which could not be accounted for merely by the volume of tissue removed in surgery. Unfortunately, there was no objective record of the size of the original insult, so it was not possible to assess these changes accurately.

The following experiment attempts to answer several of the questions posed by this prior research through a quantitative assessment of alterations in postsurgical lesion size, hemisphere size, and cellular packing densities at different intervals after neonatal cortical removal in the rabbit.

Method

Subjects. A total of 36 rabbits from 7 litters (three California, four Dutch-belted) served as subjects in this experiment. Of these, 12 animals were unoperated control subjects, 14 animals sustained small, circular, unilateral cortical lesions, and 10 animals sustained small, circular, bilateral cortical lesions at 1 day of age. They were sacrificed after varying postoperative survival periods (0, 1, 2, 4, 6, 8 days; 3, 5, 10 wks.) as shown in Table 1. A split litter technique was used for assigning subjects to lesion

TABLE 1
Progressive Morphological Changes after Neonatal Lesions

S	Race	Lesion Type	Age at Surgery	Postop. Survival
3-3	C	BC	1 Day	0 Days
4-1	D	N	"	1 Day
13-1	D	UC	"	"
4-4	D	BC	"	"
13-5	D	N	"	2 Days
13-2	D	UC	"	"
4-2	D	N	"	4 Days
5-2	C	N	"	"
13-6+	D	N	"	"
13-3+	D	UC	"	"
4-3	D	BC	"	"
14-8	C	N	"	6 Days
14-4	C	UC	"	"
14-6	C	BC	"	"
13-7	D	N	"	8 Days
13-4	D	UC	"	"
3-8	C	N	"	3 weeks
14-9	C	N	"	"
14-5	C	UC	"	"
3-6	C	UC	"	"
3-4	C	UC	"	"
1-10	D	BC	"	"
14-7	C	BC	"	"
2-6	D	N	"	5 weeks
2-7	D	N	"	"
1-2	D	UC	"	"
1-8	D	UC	"	"
2-4	D	UC	"	"
1-4	D	BC	"	"
1-9	D	BC	"	"
1-6	D	UC	"	7 weeks
1-7	D	UC	"	"
3-9	C	N	"	10 weeks
3-5	C	UC	"	"
3-1	C	BC	"	"
3-2	C	BC	"	"

Superscripts:
*M-L Hemisphere Size Measurement taken from photograph.
+Brains 8-3 and 8-6 fixed in Carnoy fluid before measurement which caused excessive shrinkage. Measurements on these Ss should be considered relative to each other only.

Other symbols:
C California albino race
D Dutch-belted race
N Normal subject
BC Bilateral cortical lesion
UC Unilateral cortical lesion
L Left hemisphere
R Right hemisphere
A-P Anterior-posterior dimension
M-L Medial-lateral dimension
D-V Dorsal-ventral dimension

TABLE 1 (Extended)

Hemisphere Size (mm)						Lesion Size (mm)				Hemisphere Vol. (cc)	
A-P		M-L		D-V		L		R			
L	R	L	R	L	R	A-P	M-L	A-P	M-L	L	R
17.5	17.5	10.0*	10.0*	13.0	13.5	3.0	3.0	3.5	3.0	1.0	1.0
						-	-	-	-		
15.0	15.5	9.0	10.0	11.5	12.0	4.0	3.0	-	-		
						4.0	4.0	3.5	3.0		
16.5	17.0	10.5	11.0	13.0	13.0	-	-	-	-		
17.0	16.5	9.0	10.5	12.5	13.0	3.0	3.5	-	-		
						-	-	-	-		
18.0	18.0	10.0*	11.0*	14.0	14.0	-	-	-	-		
17.5+	17.0+	12.0+	12.0+	13.5+	13.0+	-	-	-	-		
15.5+	17.0+	10.0+	11.0+	11.5+	13.0+	6.0	7.0	-	-		
						3.0	3.0	2.5	2.0		
21.0	21.0	15.0	15.0	15.5	15.5	-	-	-	-		
19.0	21.0	13.5	15.0	12.5	14.0	3.5	5.5	-	-		
20.0	20.0	14.0	13.5	14.0	14.0	3.0	3.0	4.0	3.5		
22.0	21.5	15.5	15.5	16.0	16.5	-	-	-	-	2.0	2.0
19.5	21.0	15.0	15.0	14.0	16.0	4.5	4.5	-	-	1.5+	2.0
27.0	27.0	13.0*	14.0*	18.0	18.0	-	-	-	-	3.5	3.5
27.0	27.0	17.0	17.0	16.0	16.0	-	-	-	-	4.0	4.0
25.0	26.5	15.0	17.0	15.5	16.0	4.0	3.0	-	-	3.0	4.0
26.0	27.0	11.0*	14.0*	16.0	17.0	6.0	4.0	-	-	3.0	3.5
25.0	26.5	11.0*	13.0*	15.5	17.0	4.5	3.5	-	-	3.0	3.5
						6.0	6.0	2.0	2.5		
25.0	25.5	15.5	14.5	15.5	15.0	3.0	1.0	3.5	1.5	3.0	3.0
28.0	28.0	16.0*	16.0*			-	-	-	-		
29.0	29.0	17.5*	17.0*			-	-	-	-		
						5.0	5.0	-	-		
						4.0	4.5	-	-		
25.0	28.5	12.0*	16.0*			4.5	5.5	-	-		
						5.0	5.5	4.0	6.0		
						3.0	3.5	2.0	2.0		
						6.0	6.5	-	-		
						4.0	4.5	-	-		
32.0	32.0	17.5*	18.0*	21.0	20.0	-	-	-	-	5.0	5.0
28.0	31.0	15.5*	17.5*	18.5	20.0	5.0	5.5	-	-	4.0	5.0
29.0	28.5	16.0*	16.0*	18.0	18.5	3.0	3.0	5.0	3.5	4.5	4.5
29.0	30.0	15.0*	16.5*	18.0	18.0	0.0	0.0	1.0	1.0	4.5	4.5

groups in all of the experiments included in this dissertation (see Appendix B for details).

Surgery. All operations were performed under light ether anesthesia using antiseptic surgical technique. The instruments, which were sterilized with a dilute (1:1000) Zephiran chloride solution during the operation, were wiped dry with a sterile gauze pad before use. The scalp was incised from a point just behind the eyes to a point just in front of the ears, with care being taken to avoid slicing through the thin skull. After the thin membranes covering the skull had been scraped away with a scalpel blade, a short (2 mm) slit was opened in the skull with the point of a sterile, disposable, 20 gauge needle. From this slit, a circular hole roughly 4-5 mm in diameter was opened in the skull with a small bone cutter. This hole was located over the posterolateral neocortex which overlies the dorsal hippocampus. The dura was then cut and a 3 mm diameter circular aspirative lesion produced, using a plastic template as a guide. The lesion was always in the left hemisphere of unilateral damaged animals in a region of cortex which divides visual and somesthetic areas (Monnier and Gangloff, 1961). In most cases the lesion extended into the lateral ventricle leaving the hippocampus exposed but intact, although in some subjects it did not fully penetrate the white matter, while in others some accidental damage of dorsal hippocampus was sustained (as revealed by subsequent histology). After the lesion was measured and photographed

the scalp was sutured with 3-0 surgical gut and the animal returned to the nest box. Throughout the operation bleeding was controlled with cotton pledgets soaked in sterile, normal saline.

Procedure. The animals were weighed at about the same time every day and all subjects except those in Group 1 were tested at least once in one or more of the behavioral studies (as noted in Appendix B). Group 1 was not tested in any of the behavioral situations. In addition, some of the subjects received injections of tritiated thymidine (^{3}H-T) at some point during the postsurgical survival period. The subjects receiving ^{3}H-T injections were used in a later autoradiographic experiment.

The animals were intracardially perfused with normal saline followed by 10% formalin after various postsurgical survival periods as shown in Table 1. The brains were then left in 10% formalin for a period of 1-5 days before being measured and photographed. The brains of the youngest subjects were particularly delicate and were left in formalin for a day or two longer than those of the older subjects in an effort to minimize the danger of damage during handling. After they had hardened somewhat the brains were removed from the formalin, and both the lesion(s) and hemispheres were measured with a pair of dividers and a metric ruler.

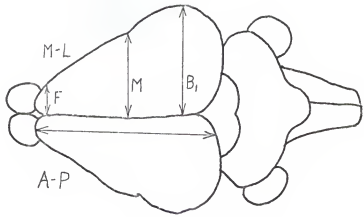
The lesions were measured both for anterior-posterior (A-P) and medial-lateral (M-L) extent. Occasionally, an annulus of slightly raised soft tissue surrounded the lesion. In this case, both measurements of the actual lesion (repre-

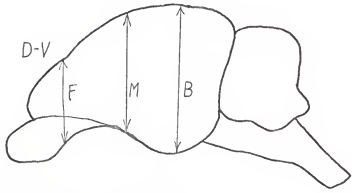
sented by a hole in the cortical surface) and of the total involved tissue (represented by the outside edge of the annulus) were recorded.

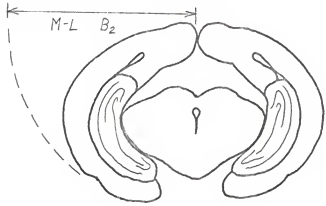
Hemisphere size was measured in three planes: anterior-posterior (A-P), dorsal-ventral (D-V), and medial-lateral (M-L). The A-P dimension was measured from the most rostral tip of the cortex (excluding olfactory bulbs) to the most caudal portion of the occipital area. The D-V dimension was measured at three levels designated back, middle, and front. The back measurement was taken at the largest part of the brain from the ventral-most surface of the temporal region (periamygdaloid area) to the dorsal-most surface of the neocortex. The middle measurement was taken just anterior to the temporal bulge, from the olfactory tubercle on the ventral surface to the dorsal-most surface of the neocortex. The front measurement was taken from the ventral surface of the olfactory tracts to the dorsal surface of the cortex just behind the rounded rostral tip (see Figure 1).

The M-L dimension was also measured at three levels: front, middle, and back. The front measurement was taken from the longitudinal fissure to the lateral edge of the cortex just behind the rounded rostral tip. The middle measurement was taken from the longitudinal fissure to the lateral edge of the cortex just anterior to the temporal bulge. The back measurement was the most difficult to make because of the fact that the rabbit brain is so rounded at this point. For this reason, it is impossible to measure

Figure 1. Diagrams showing location of gross brain measurements. A-P (anterior-posterior dimension), M-L (medial-lateral dimension), D-V (dorsal-ventral dimension). F (front measurement), M (middle measurements), B_1 (back measurement of hemisphere width as taken from photograph), B_2 (back measurement of hemisphere width as taken by second method described in text), B (back measurement of dorsal-ventral dimension).


M-L
F
M
B1
A-P

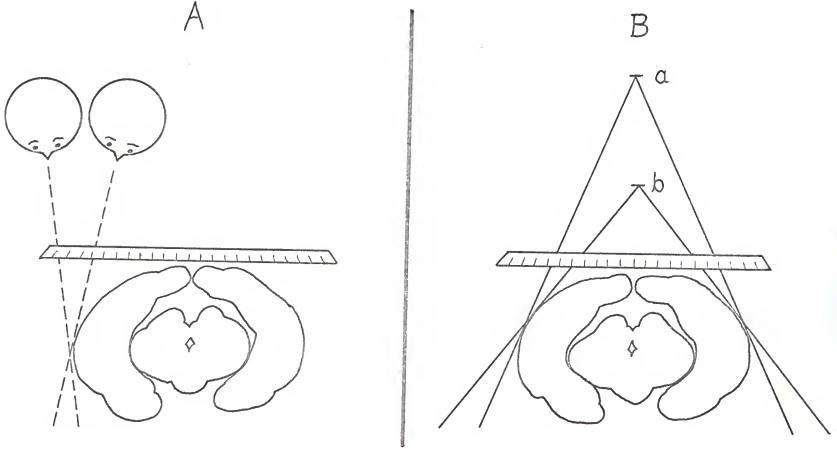

D-V
F
M
B


M-L
B2

the medial-lateral dimension directly without splitting the brain longitudinally and measuring the two hemispheres separately. It might be possible to measure this dimension directly from brain slides except for the fact that an uncertain amount of tissue shrinkage is induced in the process of histological preparation.

An indirect method of measuring the width of the hemisphere is to lay a ruler across the dorsal surface of the brain and to sight along the lateral edge of the hemisphere. However, slight variations in the position of the observer's eye will cause corresponding variations in the obtained measurements of hemisphere width as shown in Figure 2(a). Yet, by modifying this approach somewhat, this source of variation can be eliminated. If a camera is substituted for the observer in Figure 2, and if a picture is taken from directly over the midline (i.e., the longitudinal fissure), the M-L measurement can be obtained directly from the photograph (see Figure 3). As long as the picture is taken from directly over the midline the measurements obtained from the two hemispheres of one brain would be comparable. Otherwise the same basic problem illustrated in Figure 2(a) would exist and the measurements of the two hemispheres could not be compared. Also, as long as the pictures of different brains are taken at the same distance from the brains, the measurements for the two brains would be comparable. However, if the pictures are taken at different distances, the problem illustrated in Figure 2(b) would exist and the measurements

Figure 2. Diagrams of possible errors in measurement of medial-lateral dimension at back of hemisphere. "A" shows error induced by slight variations in position of observer's head when measuring by eye. "B" shows error induced by variations in distance between surface of brain and camera (or observer's eye).


A
B
a
b

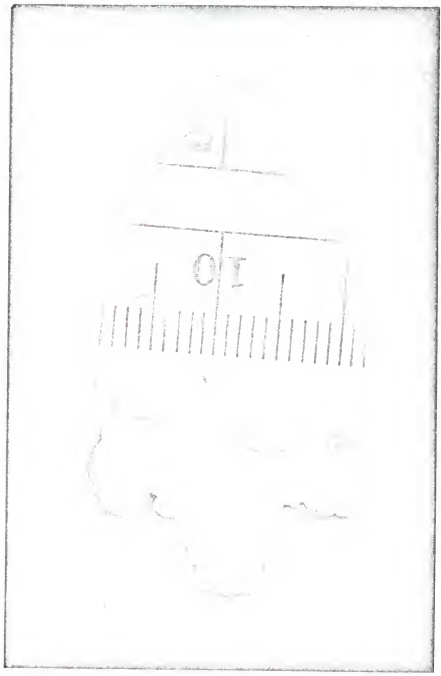


Figure 3. Photograph of rabbit brain 5 weeks after induction of unilateral neonatal cortical lesion which demonstrates one method used to measure back of hemisphere. Hippocampus can be seen filling lesion site from below.

of the two brains could not be compared. Despite these inherent limitations, this was the technique employed for Groups 2, 3, and 5. Care was taken to insure that the pictures were taken from the same height for each brain and from directly over the midline. Nevertheless the following unforeseen problem forced a change in measurement procedure for subsequent groups.

Since the damaged hemisphere was smaller in D-V extent (see results section below) than the undamaged hemisphere of unilateral damaged animals, the surface of the brain was not horizontal. That is, the brain was tilted slightly to the left. Therefore, the cortex appearing at the edge of the left hemisphere in a photograph taken from directly overhead was not homologous with the cortex appearing at the edge of the right hemisphere. The cortex appearing at the edge of the left hemisphere in the photograph was actually more dorsomedially situated than the cortex appearing at the edge of the right hemisphere. As a result, the M-L measurements obtained from the photograph were somewhat in error. They made the left hemisphere look even smaller (relative to the right hemisphere) than it actually was. This error could be reduced by supporting the damaged hemisphere with wedges of paper so that the dorsal surface of the brain was parallel with the table top. This is the technique that was used in photographing the brains of all the unilateral damaged animals throughout this study. However, the uncertainty that the brain was perfectly level (with the corresponding danger

of measurement error) led to the abandonment of this procedure of measuring the back of the brain and led to the adoption of the following technique.

One tip of the dividers was placed at the dorso-medial edge of the cortex just lateral to the longitudinal fissure. The other tip was swung downward in an arc so that it just touched the lateral-most edge of the hemisphere (i.e., the ventrolateral edge of the temporal region). Thus, the M-L measurement at the back of the hemisphere for about half of the animals in this study (Table 1) is not a pure one. It is composed of both a M-L and a D-V component (see Figure 1). Nevertheless, since it reliably reflects an actual physical characteristic of the hemisphere being measured, it is preferable to the other measurement methods mentioned above.

After the brains had been measured and photographed, the hemisphere volume of some of the brains was determined by a water displacement technique. One hemisphere at a time was immersed in water which filled a 500 ml. graduated cylinder to the 450 ml. mark. The volume of water displaced by each hemisphere was noted, and then the entire brain was immersed to check the total volume displaced against the sum of the volumes obtained for the two hemispheres. This gave a rough but direct measurement of the volume of both damaged and undamaged hemispheres.

The brains of the animals which had not received tritiated thymidine injections were then embedded in celloidin, sectioned coronally at 30 microns, and stained with thionin.

The histological treatment of the brains from animals which had received tritiated thymidine injections is described in Appendix A.

The size of the lateral ventricles, the thickness of cortex, and the thickness of white matter were determined from the stained sections of several levels of the brain. In addition, cell counts of both neurons and glia were taken in some brains, and cell packing densities and glia-neuron index were determined from these counts. The cell counts were taken at two levels of the brain approximately 2 mm lateral or 2 mm medial to the edge of the lesion in damaged hemispheres and at homologous points in undamaged hemispheres. An anterior count was taken at approximately the level of the hippocampal commissure in most brains and a posterior count at the level of the posterior arch of the hippocampus just anterior to the superior colliculi. A grid which was inserted into one eyepiece of the microscope was used for counting the cells in a 50 x 50 micron area (30 micron thick sections) just beneath the molecular layer of the cortex.

Results

The major results are presented in Table 1. This table includes only the measurements taken from the back of the hemisphere for the M-L and D-V dimensions. The measurements of the front and middle of the hemisphere are not included because of lack of space, but they will be discussed in the text below. A blank space indicates that the measurement was not taken; Groups 1 and 4 were processed histologically

before hemisphere measurements were obtained. A dash indicates that this measurement was not possible. For example, lesions were nonexistent in the right hemisphere of unilateral damaged animals or in either hemisphere of normal control animals. The superscripts are explained at the bottom of the table; superscripted measurements should be considered relative to each other only. A plus after the measurement of left hemisphere volume for S 13-4 indicates that the actual measured volume was somewhat more than 1.5 cc, but it was impossible to be more precise than that due to the imprecision of the measurement technique employed.

One apparent inconsistency in Table 1 is the size of the brain of S 3-3, an animal which died during surgery. The measured size of the brain of this day old animal is roughly equivalent to that of a 4 day old, as determined from Table 1. The most likely reason for this result is the fact that this animal was not perfused, and consequently the shrinkage normally resulting from formalin perfusion and fixation had not occurred. Another possibility is that the brain may have actually swelled beyond normal size as a result of asphyxia; the animal died of respiratory collapse created by an overdose of ether. Myers, et al. (1969), have demonstrated considerable brain swelling in some infant monkeys after experimentally induced asphyxia. Finally, the difference may represent a race difference between the brain size of California albino and Dutch-belted rabbits of comparable age, although it seems unlikely that this would account for a difference of the magnitude noted here. An ex-

amination of Table 1 is of little help in resolving this point since the majority of animals in any age group were littermates. As a result, it is impossible to compare the brain size of the two races directly within an age group. However, the brain size of the one California albino subject in the 4 day survival group is only slightly larger than that of S 3-3, and it seems quite comparable to the brain size of the Dutch-belted subjects of similar age. Also, in terms of body size the two races are quite similar for the first few weeks of life. Although the California albino subjects were slightly heavier throughout the first 3 weeks of life, the weight curves for the two races first begin to diverge after about 3 weeks of age.

An important point which should be brought out before considering the results of this experiment in detail is that the figures of Table 1 should not be construed as absolute measurements of brain size. They have not been corrected for shrinkage which undoubtedly occurred during perfusion and fixation, and the brains of different groups remained in formalin for different lengths of time (1-5 days) before measurement. In any event, the importance of these figures lies in their relation to each other rather than in their absolute value, and considered in this way they point out in a more or less objective manner some of the major consequences of neonatal brain damage on gross brain morphology.

Postoperative changes in lesion size. The initial lesions as revealed by the photographs taken during surgery (e.g., Figure 4) were very close to the intended diameter of

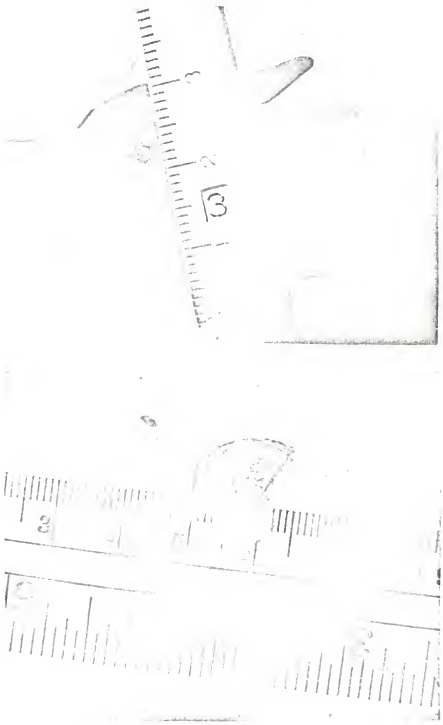


Figure 4. Photographs showing lesion size in two 1 day old rabbits immediately after surgery. Top photo is Dutch-belted subject; bottom photo is California albino subject.

3 mm and very nearly round in most cases. The actual brain lesion diameter ranged from 2.5 to 3.5 mm in different animals. During the next 8 days the mean lesion size continued to increase as shown in Figure 5. At some point between 8 days and 3 weeks the lesions of some of the animals begin to contract while others apparently continue to increase in size. In any event, 3 weeks of age seems to represent a pivotal point in the lesion size data of Table 1; some lesions are definitely smaller than the 3 mm diameter circular lesion produced in surgery while others are considerably larger. In addition, the shape of the lesions of some animals first shows a noticeable change in this age group. The most common departure from the round shape of the original lesion was an elongation in an anterior-posterior direction and/or a contraction in the medial-lateral direction. This process continued until the surface defect became little more than a slit in some animals. Figure 5 illustrates this process of initial expansion followed by subsequent contraction of the lesion. It should be noted that the apparently small lesion size of the 4 day group is probably fortuitous. This bar of the histogram is based on only one bilaterally damaged animal which had sustained very shallow lesions which did not penetrate the white matter. All of the other animals in this experiment sustained lesions which entered the lateral ventricles.

Postoperative changes in hemisphere size. The most rapid growth in the size of the brain occurred during the first week of life followed by a progressively more gradual

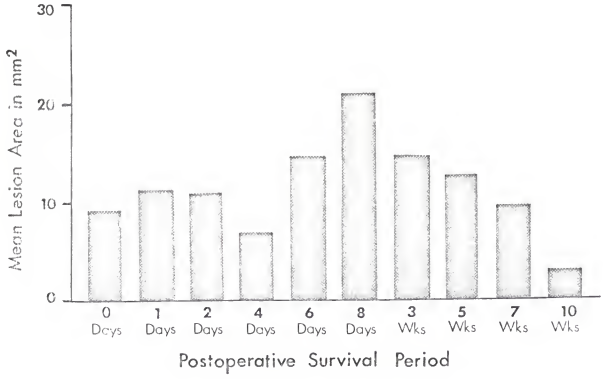


Figure 5. Histogram of lesion size at varying intervals after neonatal cortical injury. Data from subjects in which the hippocampus filled the lesion site and subjects which had developed a hydrocephalous condition are not included.

increase up to 10 weeks of age, an age at which the brain had almost reached adult proportions. Corresponding to this general increase in brain size, the measurements of the damaged and undamaged hemispheres showed progressively greater disparity (in absolute terms) during the first 10 weeks of life. In relative terms the difference in hemisphere size increased during the first week of life at a negatively accelerated rate and then remained relatively stable after that. In other words, although the difference in size between the damaged and undamaged hemispheres continued to increase as the brain grew, it was proportional to this overall increase in brain size after the first postoperative week. In terms of measured volume, the damaged hemisphere was roughly 20% smaller than the intact hemisphere of the animals sacrificed 8 days after surgery, and it remained 15-20% smaller in the animals sacrificed at longer postoperative intervals.

This decrease in volume was not caused by a decrease in size of the lateral ventricles. In fact, the ventricles were often substantially larger on the damaged side than on the undamaged side of animals sustaining unilateral lesions. This increase in ventricular size was offset by decreases in thickness of cortex, white matter, hippocampus, septum, and width of midbrain and diencephalon. In general, the degree of difference between the damaged and undamaged sides on any given measure (e. g., cortical thickness) decreased with increasing distance from the lesion site as illustrated in Figure 7. Thus, the disparity was smaller

Figure 6. Diagrams of relative differences in hemisphere size between damaged and undamaged hemispheres at three brain levels. The levels illustrated here correspond with the levels of measurement listed in Table 2. The broken lines indicate the size of the damaged hemisphere in 3 week old subjects which had not developed a hydrocephalous condition. For this reason, the relative size difference illustrated here is somewhat greater than the mean difference for all subjects as listed in Table 2. The difference is greatest at the level of the lesion (center diagram) and decreases with progressively greater distance rostrally and caudally from the lesion site. It is also somewhat greater in the dorsal regions than in the ventral regions of the hemisphere.

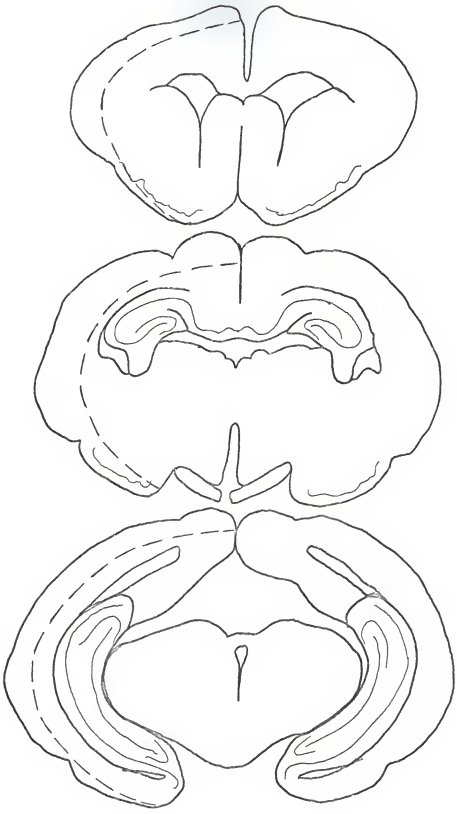


Figure 7. Photographs at three levels of rabbit brain showing differences in development of damaged and undamaged hemispheres 3 weeks after unilateral neonatal insult. Note differences in width of diencephalon (b) and midbrain (c) and difference in anterior-posterior extent of hippocampus. Thionin stain, 12.5 X.

A

B

C

at progressively more anterior or posterior levels and somewhat smaller in the ventral portion of the hemisphere than in the dorsal. Statistically significant differences extended over considerable distances from the lesion site as shown in Table 2, which is based on an analysis of the subjects in the 3 week survival group. The damaged side of subjects with unilateral lesions did not differ significantly from the two damaged sides of subjects with bilateral lesions, and the intact side of unilateral damaged subjects did not differ from the intact hemispheres of normal subjects on any of the measures taken. All measurements were obtained from the calibrated stage of the microscope.

Postoperative changes in microstructure. The cell counts performed on four subjects in the 5 week survival group (2 with unilateral damage, 2 with bilateral damage) and two unilateral damaged subjects in the 7 week survival group revealed an increase in cell packing density both for neurons and glia and a large increase in glia-neuron index in the damaged hemispheres (as compared with the intact hemispheres). The anterior and posterior counts were roughly comparable, and the only noticeable difference between the two age groups was an increase in glial packing density, along with the corresponding increase in glia-neuron index, in the older animals. The only noticeable difference between the damaged hemispheres of unilateral damaged subjects and bilateral damaged subjects of the same age was a higher glia-neuron index in the bilateral damaged subjects (.98 vs .75).

TABLE 2

Differences in Mean Size of Several Brain Structures Located in Damaged or Undamaged Hemispheres of Subjects Surviving for 3 weeks Postoperatively

Brain Level*	Brain Structure	Mean Size Undamaged Side	Mean Size Damaged Side	Differences	% Differences	t**	p <
Anterior	Cortical Thickness	2.27 mm	2.09 mm	.18 mm	8.0	2.67	.01
	Septum width	1.70 mm	1.53 mm	.17 mm	10.0	3.29	.005
	Hemisphere width	7.93 mm	7.23 mm	.70 mm	8.9	4.61	.001
Middle	Cortical Thickness	1.76 mm	1.49 mm	.27 mm	15.3	8.23	.001
	Diencephalon width	5.01 mm	4.53 mm	.48 mm	9.6	8.32	.001
	Hemisphere width	9.87 mm	9.54 mm	.33 mm	3.3	1.83	.05
Posterior	Cortical Thickness	1.39 mm	1.26 mm	.13 mm	9.4	3.25	.005
	Hippocampal Thickness	2.30 mm	2.03 mm	.27 mm	11.7	6.45	.001
	Midbrain width	5.37 mm	5.00 mm	.37 mm	6.9	8.81	.001
	Hemisphere width	10.59 mm	10.24 mm	.35 mm	3.3	6.26	.001

* Brain levels used were identical to those pictured in Figure 7.

** $\underline{df}$ = 12

On the basis of the very limited cell counts reported here, it is difficult to say whether or not this represents a real difference in the reactions of the brain of unilateral vs bilateral damaged animals to the insult. Similarly, it is impossible to say with certainty whether or not the observed increases in neural and glial packing density are due merely to a proportionate decrease in hemisphere volume or if they might be due, in part, to increased or prolonged neurogenesis or gliogenesis stimulated by the lesion.

Discussion

Changes in lesion size. One of the most disturbing aspects of the data presented in Table 1 is the extreme variability in the size of the lesions of different animals and sometimes even between the two lesions in the same bilaterally damaged animal (e.g., S 1-10). Some of this variability may have resulted from differences in the degree of disruption of local blood supply; bleeding during surgery was definitely more pronounced in some subjects than in others. However, two other factors seem to account for most of the variability: 1) the development of a hydrocephalous condition in about 20% of the animals and 2) a tendency for the dorsal hippocampus to push up and fill the lesion site in some subjects.

The hippocampus was most likely to fill the lesion when the defect was immediately over the most dorsal aspect of the hippocampus and when some accidental damage to the hippocampus had been produced. A particualrly striking ex-

ample is S 13-3 which is pictured in Figure 8. At the level of the lesion the hippocampus can be seen growing up through the cortical defect in an extremely bizarre manner. This abnormal appearing hippocampal tissue continues anteriorly over the surface of the cortex, and in the most anterior sections pictured (Figure 8a, b) two layers of hippocampal tissue can be seen separated by a normal appearing layer of cortex. Due to this overlap, the apparent size of the cortical defect, as listed in Table 1, was much larger than the actual defect as revealed in the brain sections. Figure 9 shows the surface of the brain of this subject and the appearance of the lesion site at the time of measurement. A less striking example is the brain of S 2-4 pictured in Figure 3. This subject had not sustained any hippocampal damage and the smooth surface of the hippocampus can be seen filling the cortical defect.

The question of whether the hippocampus filled the lesion site of some animals because the lesion had not filled in or whether the lesion did not fill in because of a physical barrier created by the hippocampus is an academic one at this point; but it seems likely that both of these situations may have existed in the same animal. That is, initially the hippocampus may have filled the lesion site because of the existence of the cortical defect and because of physical stresses and pressures created by rapid growth and expansion of the structure. Having done so, this might have prevented the subsequent contraction of the edges of

Figure 8. Photographs of bizarre development of hippocampus in 4 day old unilateral damaged rabbit. Top photos of damaged hemisphere (a & b) show a layer of cortex separating two layers of hippocampal tissue, an abnormal rostral extension above the surface of the cortex and a diminutive dorsal hippocampus in its usual position. The two middle pictures show the hippocampal tissue growing through the lesion site and connected with the diencephalon by a stalk of fimbrial fibers (c) and highly vascularized glia-connective tissue (d). The bottom photos show a small and abnormally shaped posterior-ventral hippocampus with an unusual extension of neural tissue growing through the lesion site (e). This extension has many of the cytoarchitectural characteristics of normal subiculum. Mayer's Hematoxylin, 12.5 X.

a

b

c

d

e

f

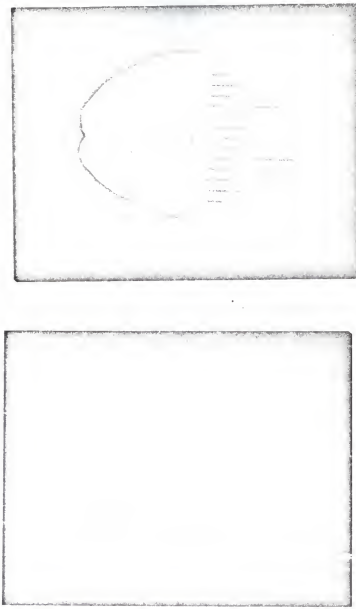


Figure 9. Photographs of brain of S 13-3 showing abnormal tissue growing out of lesion site.

the lesion which would have occurred if the hippocampus had been absent from the lesion site.

It is possible that the continued growth of the hippocampus within the confines of the lesion in some of the older subjects may have actually caused a prolonged expansion of the lesion rather than merely preventing contraction. For example, consider the extremely large lesion size of S 1-10, 1-6, and 3-5 in the left hemisphere. However, it is unlikely that this had any influence on the initial expansion of the lesion for the first 8 days postoperatively since none of the subjects sacrificed before 3 weeks of age showed any plugging of the lesion site by the hippocampus (except for S 13-3 which had apparently suffered hippocampal damage during surgery). The reasons for this initial expansion of the lesion are uncertain and probably multiple, but the relatively long time course suggests the participation of a mechanism which might lead to progressive cell death over a period of several days such as interruption of local blood supply.

In a recent study of morphological changes after electrolytic lesions of caudate and hypothalamus of rats, Wolf and DiCara (1969) report a similar sequence of lesion size changes. The apparent lesion size increased rapidly during the first postoperative day and reached a peak at some point between 1 day and 1 week postoperatively. This was followed by a subsequent progressive contraction of the lesion for the next 15 weeks postoperatively. The mechanisms involved in producing the lesion size changes observed in these two

studies are almost certainly different. In particular, the very rapid expansion of the apparent lesion size during the first postoperative day in Wolf and DiCara's (1969) study was due to the disintegration of cells which had been "fixed" by the current but which appeared normal at 1 hour postoperatively. However, the major implication of their study seems to apply equally well to this experiment; namely, a reconstruction of extent of tissue damage on the basis of the apparent boundaries of the lesion would vary widely depending on the length of the postoperative interval.

Changes in cerebral hemisphere size. The reduced size of the damaged hemispheres of animals receiving neonatal cortical lesions in this experiment is illustrated diagrammatically in Figure 6 and photographically in Figure 7. The reduction was greatest in the immediate area of the lesion, but it extended over considerable distances in all directions from the lesion site and included many structures which were not directly involved in the lesion. This apparent stunting of growth of many structures over widespread areas of the hemisphere is undoubtedly a complex process dependent on a number of more or less interdependent mechanisms. For example, intracranial injury in rabbits is accompanied by swelling of neuronal mitochondria and by disruption of the associated process of oxidative phosphorylation (Tigranyan, 1968) over wide areas of the brain. Similarly, Guth and Watson (1968) report increases in cerebral glycogen content after a puncture wound in the brain of the rat. This re-

action is greatest in the immediate area of the lesion but also extends over considerable distances from the lesion site. Both of these metabolic disturbances, however, might be secondary to a disruption of blood supply produced by the brain injury and to the ischemia, anoxia, hypercapnia (buildup of carbon dioxide level), and drop in pH resulting from circulatory failure. Any long term disruption of blood supply to the brain of the neonate would almost certainly take its toll on the subsequent development of the brain. Therefore, Kosmarskaya and Palenova's recent (1968) demonstration that closed cranial injury in 5 day old rabbits inhibits development of the venous system of the brain is of considerable importance for interpreting the results of this experiment. In brief, they showed that both veins and arteries were significantly contracted over the entire cerebral hemisphere during the first 4 hours after the trauma, although the contraction was most severe in the immediate vicinity of the trauma. By the sixth day after trauma the surface arteries of the hemisphere had begun to increase in diameter, but they never did reach normal dimensions. Furthermore, formation of the large veins and their branches was still occurring in experimental animals, whereas this process was complete in normal animals of the same age (11 days). By the 21st day after trauma the surface arteries of experimental animals were still smaller and more irregular in diameter than normal, and the formation of the venous system was still occurring, although formation of the venous

system of control animals was essentially complete at this age (26 days) and looked identical to the adult pattern.

On the basis of the limited number of cell counts reported in the present experiment, the reduced hemisphere volume produced by neonatal cortical lesions apparently does not depend on a loss of cells. It is also not dependent on decreased ventricular volume. Instead, some other factor(s) such as decreased cell size, decreased axonal and dendritic field, decreased glial process field, or decreased intercellular space must be involved. Considering the increased number of glia cells in the damaged hemispheres of the experimental animals, however, it seems somewhat unlikely that a decrease in glial process field could account for the reduced hemisphere size. Such a contraction of glial porcesses may indeed have occurred, but any such reduction would be at least partially offset by the observed increase in number of glia. This and several other bits of evidence seem to implicate a reduction in extracellular space or a decrease in axonal and dendritic field or both.

Before 5 days of age, large but variable extracellular spaces can be seen in electron micrographs of normal rabbit brain (Smith, 1963). These spaces are largest in those regions which are destined to become white tracts and become obliterated after 5 days of age by the close apposition of processes and cells. This evidence supports the observation of Schadé and Baxter (1960) and Schadé, Vanbacker, and Colon (1964) that the major growth in apical and basal

dendrites in rabbit cortex occurs from the 5th to the 15th day of life, which is roughly the time period during which the major difference in size between the damaged and undamaged hemispheres developed in the present study. Furthermore, in an electron microscopic study of rat cerebral cortex, Caley and Maxwell (1970) reported an increase in extracellular space through the fifth day of age, the period of major axonal and dendritic growth. These large spaces disappeared rapidly after 5 days of age concommitantly with the development of the complex interrelations between axons, dendrites, glia, and blood vessels. They hypothesize that the presence of these large extracellular spaces might be particularly conducive to the growth of cell processes during a time when there are very few blood vessels (Caley and Maxwell, 1968a). If this hypothesis is correct, then any manipulation leading to premature collapse of these extracellular spaces might cause a stunting of growth of cellular processes.

Van Harreveld, Crowell, and Malhotra (1965) have presented evidence that the lack of extracellular space usually observed in electron micrographs of adult brain tissue may be due at least partially to anoxia during fixation. Although the newborn is generally considered to be less sensitive to anoxia than the adult (but see Hicks, 1968), chronic anoxia created by a disruption of blood supply development such as that reported by Kosmarskaya and Palenova (1968) after early brain insult might cause a reduction of

extracellular space which is not seen after the comparatively brief anoxia occurring during fixation. This reduction of extracellular space might then hinder the development of cell processes and lead to a reduction in hemisphere size. It is of interest here that Hicks, Cavanaugh, and O'Brien (1962) found a permanent stunting of dendrites in cortical pyramidal cells after infant rats were asphyxiated for 30 minutes but subsequently revived.

As noted earlier, disruption of blood supply does more than just produce anoxia. For this reason, the mechanism proposed above is probably a gross oversimplification of the process(es) leading to reduced hemisphere size after neonatal brain injury. Nevertheless, it has the advantage of being directly and fairly easily testable. It seems that the logical next step would be a combined electron microscopic and Golgi study of infant brain tissue after neonatal insult.

Experiment 2: Neonatal Cortical Insult without Tissue Removal

In an effort to test the suggestion presented in the discussion of the previous experiment that the decreased hemisphere size resulting from neonatal cortical insult might be caused by a disruption of blood supply, three animals were subjected to a surgical procedure which would disrupt blood supply in one hemisphere without actually removing tissue surgically. Although a positive result (i.e., reduced hemisphere size) would not prove that disruption of blood

is sufficient by itself to produce the effects noted previously (i. e., other factors could be involved as well), a negative result would suggest that disruption of blood supply is not sufficient in itself to produce the effect. Instead, it would show that actual tissue removal, with or without the accompanying blood supply disruption, is also necessary to produce the observed disturbance in cerebral development.

Method

Three one-day-old California albino rabbits were subjected to the same surgical procedure as described in Experiment 1 up to the point of tissue removal by aspiration. At this point, the beveled tip of a sterile disposable 22 gauge hypodermic needle was used to produce tears or slits in the cortex of one hemisphere 2-3 mm deep, a depth which should have penetrated the lateral ventricle. One subject received two semicircular slits oriented medial-laterally but separated by a 1 mm bridge of intact tissue at top and bottom (()). A second subject received two similar slits oriented anterior-posteriorly, and separated by a 1 mm bridge of tissue at front and back (⊃). The third subject received a slit which formed a complete circle, thus creating a circular island of cortical tissue (o) approximately 3 mm in diameter. These subjects were sacrificed 3 weeks after surgery along with several of their littermates which had received aspiration lesions on the same day. Handling, behavioral testing, histological procedures, and

measurement techniques for these animals were the same as those described for their littermates in the previous experiment.

Results and Discussion

The major results are presented in Table 3. The first feature to be noted is the fact that all three brains displayed definite lesions or holes in the cortical mantle which extended into the lateral ventricle. Thus, although no tissue had been removed during surgery, the tissue in the immediate region of the needle cuts (presumably inside them) had degenerated during the postoperative interval. The size of these lesions was somewhat larger than those of their littermates which had survived for the same length of time after aspiration-produced lesions (see Table 1 for comparison), but this is probably due to the fact that the size of the affected area was more difficult to control using this technique and more tissue may have been included within the confines of the needle cuts than was removed by aspiration in the animals of Experiment 1. Alternatively, this may represent a real difference in the reaction of the brains to the two types of injury. The presence of the isolated and subsequently degenerating tissue within the needle cuts might conceivably have caused a greater inflammatory reaction of adjacent tissue, perhaps leading to greater neural degeneration than that caused by surgical tissue removal.

One indication that the reaction of the brain to this type of injury may be more severe than to surgically induced

TABLE 3

Morpohlogical Changes after Neonatal Damage Without Tissue Removal

S	Race	Lesion Type	Age at Surgery	Postop. Survival	Hemisphere Size (mm) A-P L	A-P R	M-L L	M-L R	D-V L	D-V R	Lesion Size (mm) L A-P	L M-L	R A-P	R M-L	Hemisphere Volume (cc) L	R
14-1	C	UVNC	1 Day	3 wks	24.0	25.5	14.0	15.5	14.5	16.0	5.5	4.0	-	-	3.0	3.5
14-2	C	UHNC	1 Day	3 wks	25.5	26.0	14.0	17.0	15.0	16.0	4.5	3.5	-	-	3.0	4.0
14-3	C	URNC	1 Day	3 wks	25.0	25.5	15.0	15.0	16.0	16.0	5.5	5.0	-	-	3.5	3.5

Symbols:
- UVNC Unilateral vertical needle cuts (())
- UHNC Unilateral horizontal needle cuts (⊃)
- URNC Unilateral round needle cut (o)
- C California albino race
- L Left Hemisphere
- R Right Hemisphere
- A-P Anterior-posterior dimension
- M-L Medial-lateral dimension
- D-V Dorsal-ventral dimension

tissue removal is the fact that the intact hemisphere in these animals was generally smaller than the intact hemisphere(s) of their unilateral damaged or normal littermates from Experiment 1. Since the differences are small and based on only a few animals, this effect may be more apparent than real. Nonetheless, the possibility remains that the developing brain reacts more severely to this type of insult than it does to aspirative lesions and that this reaction produces a fairly widespread stunting of growth reaching even into the undamaged hemisphere.

An apparent discrepancy in Table 3 is the fact that the dimensions of the two hemispheres in S 14-3 are almost identical. This resulted from an enlargement of the lateral ventricle in the damaged hemisphere which offset the smaller thickness of cortex, hippocampus, and brain stem as revealed by the brain sections. Although this hydrocephalous condition may have contributed to the thinning of cortex and hippocampus, it probably played a minor role, if any, since the extent of thinning was comparable to that observed in the unilateral damaged subjects without hydrocephalous. Furthermore, close examination of the ventricular lining revealed no stretching or splitting of the ependyma which accompanies neural compression and destruction in more severe hydrocephalous in rabbits (Weller and Wisniewski, 1969).

Experiment 3: Morphologic(al) Effects of Postnatal Cortical Damage

The results of Experiment 1 showed that neonatal cortical destruction in rabbits leads to a subsequent reduction in size of the damaged hemisphere when compared with normal or undamaged hemispheres. However, this fact by itself says nothing about the possible importance of the age of the animal at the time of surgery. That is, it does not answer the question of whether lesions sustained at later ages might also produce a decrease in hemisphere size relative to normal or whether this effect is specific to very early damage. The hypothesis presented in Experiment 1 that the reduced hemisphere size is caused by a stunting of axonal and/or dendritic growth implies that this difference in hemisphere size is probably specific to damage sustained during the early postnatal period when the major growth of axons and dendrites occurs.

Another unanswered question is whether or not the decrease in lesion size after neonatal brain damage might be a function of the age of the animal at the time of the insult. The results obtained by Isaacson, et al. (1968) with cats suggested that the degree of reduction in lesion size might be greater the earlier the lesion is induced. However, since their animals with early lesions usually survived for longer postoperative periods than those with later lesions, it was not possible to say with certainty whether this effect was due to the age of the animal at the time of surgery or to the length of the postoperative interval.

The present experiment attempts to answer these questions by subjecting ten rabbits to carefully controlled neocortical lesions at different ages and sacrificing them after varying postoperative intervals.

Method

Subjects. Ten animals (six California albino, four Dutch-belted) served as subjects in this experiment. One was an unoperated control subject. The remainder received small circular cortical lesions (3 mm in diameter) in one or both hemispheres at varying ages (1, 6, 9, 11 weeks) according to the schedule shown in Table 4. They were sacrificed after varying postoperative intervals as shown in Table 4. The three animals which received lesions at 1 week of age were experimentally naive at the time of surgery. They were subsequently tested in a series of behavioral situations described in Chapter 2 (see Appendix B for details). The subjects receiving lesions at 6 weeks of age or later had previously served as normal control subjects in a heart-rate habituation study.

Procedure. The surgical technique for the 1 week old animals was identical to the one described previously in Experiment 1. There were two changes in surgical procedure for the older animals: (1) the animals were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally), and (2) the skull was opened with a dental burr and the hole enlarged to 4-5 mm diameter with rongeurs. Histological procedures and measurement techniques for all these animals were

TABLE 4

Progressive Morphological Changes after Postnatal Lesions at Various Ages

S	Race	Lesion Type	Age at Surgery	Postop. Survival	Hemisphere Size (mm) A-P L	A-P R	M-L L	M-L R	D-V L	D-V R	Lesion Size (mm) L A-P	L M-L	R A-P	R M-L	Hemisphere Volume (cc) L	R
10-2	C	BC	1 wk	5 wks	27.5	27.0	18.0	18.0	20.0	20.0	4.0	4.0	2.0	3.0	4.5	4.5
9-1	C	UC	1 wk	7 wks	30.5	31.0	20.0	20.0	21.0	21.0	5.0	3.0	-	-	5.0-	5.0+
9-2	C	UC	1 wk	7 wks	27.0	29.0	18.0	20.0	20.0	21.0	3.5	3.0	-	-	4.0+	5.0
5-4	C	N	6 wks	14 wks	32.5	32.5	21.0	21.0	20.0	20.0	-	-	-	-		
5-10	C	UC	6 wks	14 wks	32.0	32.0	20.5	21.0	21.0	21.0	2.0	2.0	-	-		
5-1	C	BC	6 wks	14 wks	30.5	31.5	20.5	21.0	22.0	22.5	2.5	2.5	1.5	2.0		
8-1	D	UC	9 wks	3 wks	28.5	29.0	19.5	19.5	21.0	21.0	3.0	3.0	-	-		
8-5	D	UC	9 wks	3 wks	30.0	30.0	20.0	20.0	21.0	21.0	3.0	4.0	-	-		
6-1	D	UC	11 wks	5 wks	30.5	31.0	20.0	20.0	21.0	21.5	2.5	3.0	-	-		
6-8	D	UC	11 wks	5 wks	30.5	30.5	19.5	20.0	21.0	21.0	2.5	3.0	-	-		

Symbols:

- C California albino race
- D Dutch-belted race
- N Normal subject
- BC Bilateral cortical lesion
- UC Unilateral cortical lesion
- L Left Hemisphere
- R Right Hemisphere
- A-P Anterior-posterior dimension
- M-L Medial-lateral dimension
- D-V Dorsal-ventral dimension

the same as those described in Experiment 1.

Results

The results are presented in Table 4. The first feature to be noted is that the damaged hemispheres of two of the subjects (10-2, 9-12) which received lesions at 1 week of age are smaller than the undamaged hemisphere of S 9-2 and smaller than the undamaged hemispheres of similar aged subjects in Experiment 1 (see Table 1 for comparison). However, the damaged hemispheres in these two subjects were not as small as the damaged hemispheres of similar aged subjects in Experiment 1. In other words, although cortical damage at 1 week of age produced a stunting of hemisphere growth this stunting was not as severe as that produced in Experiment 1 by neonatal damage of the same absolute magnitude. Subject 9-1 developed a rather severe hydrocephalous condition bilaterally, and it is interesting that both hemispheres are somewhat larger than normal.

In subjects receiving lesions at 6 weeks of age or later the damaged and undamaged hemispheres were almost identical in size. The damaged hemisphere was very slightly smaller on one dimension or another for some subjects, but there were no statistically significant size differences ($p < .1$) between the damaged and undamaged hemispheres on any dimension.

As in Experiment 1, the lesion size data were rather variable and therefore rather difficult to interpret. However, most of the variability seems to be attributable to the same two factors considered in Experiment 1; namely, S

9-1 developed a hydrocephalous condition (as noted previously), and the hippocampus partially filled the lesion site in the left hemispheres of both Ss 10-2 and 9-2. The fact that the hippocampus filled the lesion site of these animals to a lesser degree than it did in many of the neonatal damaged animals of Experiment 1 is probably significant, particularly when one considers the fact that the hippocampus showed absolutely no sign of pushing into the lesion site of animals receiving lesions at 6 weeks of age or later. It seems reasonable that the hippocampus would be most likely to fill the void created by a cortical lesion when it is in a state of rapid growth and development and least likely to fill the void when it and the rest of the brain have reached full adult proportions and are no longer increasing in size.

In any event, if one eliminates the lesion-size data for the left hemispheres of Ss 9-1, 9-2, and 10-2 (for the reasons mentioned above) a very orderly pattern emerges. Considering all of the animals together (i.e., ignoring age at the time of surgery) the size of the lesion decreases progressively with increasing postoperative interval. Furthermore, the lesion size at any particular postoperative interval is quite comparable to that of Experiment 1 for the same interval (see Figure 5 for comparison). This suggests that the observed reduction in lesion size over time is primarily a function of the postoperative interval. It does not seem to depend to any great extent on the age of the subject at the time the lesion was induced unless the earli-

ness of the lesion leads to the formation of a physical barrier such as hydrocephalus or a hippocampus filling the lesion site.

Discussion

The observation that brain damage induced in 1 week old rabbits leads to a stunting of hemisphere growth but that this stunting is less severe than that produced by neonatal brain damage fits the hypothesis presented in Experiment 1 that the reduced hemisphere size is due to a decrease in axonal and dendritic field or extracellular space or both. As noted previously, the major growth of apical and basal dendrites in rabbit cortex takes place between the 5th and the 15th day of life (Schadé, et al., 1964), and the extracellular spaces which are prominent before day 5 become obliterated by the subsequent rapid growth of cellular processes (Smith, 1963). Although this correlation is suggestive, it is very inconclusive in the absence of data from rabbits suffering brain damage between the ages of 1 and 6 weeks. Schadé, et al. (1964) report that the major growth in basal dendrites occurs between days 5 and 15; whereas the major growth of apical dendrites occurs between 10 and 15 days of age. By day 30 both apical and basal dendrites have almost reached adult proportions. Based upon this scheme then, the obvious followup of this study, but one which will have to await future research, is to subject young rabbits to carefully controlled lesions of the cortex at various ages during the first month of life, perhaps 5, 10, 15, and 30 days of age.

CHAPTER 2:
DEVELOPMENT OF PERCEPTUAL AND MOTOR CAPACITIES

Experiment 1: Orientation Toward and Return to Nest

Schneirla (1965) has proposed that the development of species-typical distance modality functioning (i.e., orientation and perception) and the subsequent utilization of distal sensory cues in later developmental stages is dependent on the prior development of the proximally based sensory mechanisms of somesthesis and chemoreception. To the extent that this postulate is true, any disruption of an animal's ability to learn the sensory characteristics of the nest situation or to develop an organization of behavior based on the proximal stimuli which are normally present during the early stages of an animal's postnatal life should have serious consequences for its later perceptual and social behavior. Two such disruptive events might be: (a) sensory deprivation, as imposed either by removing the subject from the litter or nest or by changing the sensory characteristics of the nest-litter situation, and (b) neurological damage.

Several studies have demonstrated the adverse effects of the first type of disruptive event. Rosenblatt, Turkewitz, and Schneirla (1962) removed kittens from the litter at different ages, raised them in a brooder for

various lengths of time, and then returned them to the litter. Those kittens which were removed from the litter at the earliest postnatal times were much more impaired in finding the nipple and in their suckling behavior, as well as their relationships with mother and littermates than were kittens removed at a later age but raised in the brooder for an equal length of time. Similarly, rat pups which grow up in an unstable environment in which the olfactory and visual characteristics change every 3 days never develop the normal tendency to orient towards and return to the nest region after being placed in different parts of the cage (Turkewitz, 1967). Finally, in a very well known series of studies, Harlow (e.g., Harlow, 1960) has demonstrated the devastating effects of raising infant primates in isolation from their natural mothers and siblings and in the absence of normally occurring somaesthetic stimuli on the subsequent development of perceptual, emotional, and social behavior.

The following study is an attempt to assess the influence of the second factor, namely, neurological damage, on the development of the infant rabbits' ability to orient toward and return to the nest when removed to a distant point in the nest box.

Method

Subjects. A total of 49 rabbits [Orychtolagus cuniculus (L.)] from eight litters (three California, five Dutch-belted) served as subjects. Of these, 13 animals were unoperated controls. The remaining 36 animals had

suffered some type of brain lesion at one day of age according to the following scheme: 12 with bilateral hippocampal damage, seven with large bilateral cortical lesions (controls for hippocampal damaged subjects), seven with small round bilateral cortical lesions (as previously described in the anatomical studies), and ten with small round unilateral cortical lesions.

Surgery. The surgical technique employed for the subjects with small cortical lesions has been described previously in the anatomical studies. The technique for the hippocampal damaged and cortical control subjects was almost identical but differed in the following respects. The skull defect was enlarged considerably in dorsal and ventral extent, extending almost to the midline dorsally and roughly to the middle of the temporal region ventrally. The dura was then cut and the hippocampus exposed by gentle aspiration of the overlying neocortex. The exposed portions of the hippocampus were then removed, with little attempt being made to remove the most dorsal and ventral aspects. After exposing the hippocampus in the cortical control subjects, the cortical lesion was enlarged slightly in an attempt to control for any possible mass action effects. Bleeding was controlled with cotton pledgets soaked with sterile, normal saline.

Procedure. A staggered longitudinal design was used with litters beginning testing on the first (2 litters), second (2), third (2), fourth (1), and fifth (1) days of

life . Testing was concluded on the seventh day of life. At roughly the same time every day, after being weighed, each subject was tested for its ability to return to the nest within 30 sec. of being placed in the nest box. On each of two trials the subject was placed at the end of the nest box farthest from the nest. At the beginning of Trial 1 the subject was oriented directly away from the nest, and on Trial 2 directly toward the nest. In addition to scoring each trial for a "return" or "no return" the general behavior of the subject was noted. Figure 10 , in which a 5 day old Dutch-belted rabbit is returning to the nest, illustrates the testing situation.

Results and Discussion

Despite the rather dramatic morphological changes observed in the anatomical study, there were no behavioral effects in this study arising from the brain damage. None of the lesion groups differed significantly from each other or from the normal control group. For this reason the data for all lesion groups are presented together in Figure 11.

The behavior of the animals in this experiment can be divided roughly into three stages. During the first three days of age the animals showed very little evidence of any orientation toward the nest. They floundered around, often in large circles, until encountering an obstacle such as the metal wall of the nest box or the wall of tightly packed straw around the outside of the nest. At this point they burrowed into the straw and appeared to go to sleep. When

Figure 10. Five day old Dutch-belted rabbit returning to nest.

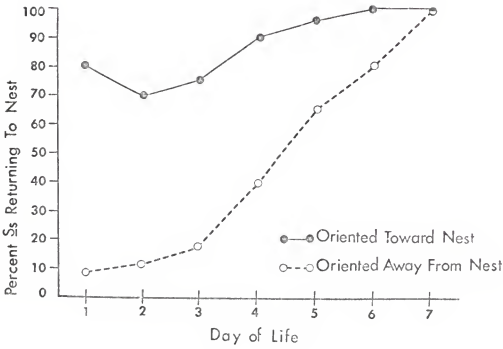


Figure 11. Percentage of subjects returning to the nest as a function of age. Subjects were oriented away from the nest before trial 1 (open circles) and toward the nest (filled circles) before trial 2.

a subject made its way into the nest, as it often did when oriented toward the nest at the beginning of the trial, it seemed to be merely by chance.

From day four to about day seven there was a progressive improvement in the ability of the animals to orient towards and find their way into the nest. Unlike their behavior during stage one, the animals would often stop and swing their heads from side to side (apparently sniffing the straw or the side of the nest box) before proceeding on. Their behavior rather abruptly took on the appearance of seeking or "appetitive" behavior (Hinde, 1966). This rather abrupt change in behavior which occurred between days three and four is reflected in Figure 11 as an inflection in both curves.

Although no attempt was made to experimentally determine the sensory cues which a subject used to find the nest, a number of different factors seemed to be involved. The first, and perhaps most important, is the apparent use of olfactory cues. As mentioned above, the subjects seemed to be sniffing the straw and the side of the nest box as they approached the nest. This was especially true on the fourth and fifth days when the animals were particularly prone to follow along the wall of the nest box. In view of this, it is conceivable that the mother may have "marked" the wall of the nest box with the submandibular glands under the chin which the rabbit apparently uses for territorial marking in the wild (Mykytowycz, 1965). However, this behavior was

never observed in any of the females while they were in the nest. It is also possible that an odor trail may have built up as the female inadvertently brushed against the walls of the nest box while nursing her pups.

Another bit of evidence suggesting the importance of olfactory cues is the disruption of behavior observed in one litter which was placed in a clean nest box on day six after the female had fouled the old nest. The effect was particularly striking since every subject had performed perfectly on both trials the day before. This also agrees very well with the results of Turkewitz (1967), who reported a total loss of orientation to the nest region in a group of rats raised in a cage whose olfactory and visual characteristics were changed every three days. This change in behavior occurred after the first olfactory change.[1]

It is quite possible, however, that the change in physical characteristics of the nest also played a large, if not major, role in producing the disorientation observed in the present study. There is little question that the

[1]Although proving nothing with regard to the question of the role of olfactory cues in the development of nest orientation, it is interesting to note that the day to day improvement in performance observed in this study very closely parallels the ontogenetic development of mitral cell synapses within the "olfactory analyser" as reported by Pilipenko (1968). The fact that these anterio-ventral brain areas (olfactory bulb, prepyriform cortex, amygdaloid nuclei, and periamygdaloid cortex) are precisely the areas which showed the least change (i.e., shrinkage) in the preceeding anatomical studies may be of some consequence for interpreting the absence of lesion effects reported below.

nature of the nest and the firmness of the bed of straw in the nest box had an effect on the performance of the young rabbits. The subjects moved fastest over firmly packed straw and were most likely to find their way into a shallow nest; whereas they were least likely to return to a well hollowed nest with steeply sloping sides and moved slowest, or not at all, in loosely packed straw (see Ross, Sawin, Zarrow, and Denenberg, 1963, for description of nest types). In very loose straw, many animals showed a strong tendency to burrow and go to sleep. At least a portion of the observed improvement in performance across days, therefore, may be due to the fact that the bedding straw became progressively more firmly packed and the boundaries of the nest progressively less distinct over time.

One indication of the possible strength of this factor is the performance of one group of rabbits (whose data are not included in the analysis) tested for the first time at 2 days of age. Every subject in this group found its way into the nest by following the same route along one side of the cage through a natural tunnel in the straw which opened into one side of the nest. When the last few inches of the tunnel were collapsed, most of the subjects found their way into the nest by burrowing through the straw. Thus, when physical obstacles are removed and a physical guide (in the form of the tunnel along a wall) is provided, the rabbits were able to return to the nest at a much earlier age than they would have been able to do under normal testing condi-

tions. However, one should not conclude from this that the physical characteristics of the nest and its surrounds are the only factor, or even the most important factor, determining the infant rabbits' ability to orient toward and return to the nest. These subjects still showed no evidence of orienting toward the nest. On the contrary, they seemed merely to be following the path of least resistance and ended up in the nest almost by accident. (cf. the behavior of the other groups during stage 1).

A third factor which seemed to influence the performance of the animals in this experiment was the presence or absence of littermates in the nest. Confirming Turkewitz' (1967) results with infant rats, the rabbits seemed to move more energetically and over greater distances and they were more likely to find the nest during the 30 sec. of each trial if littermates were present in the nest. A number of possible cues from the litter may have played a role including olfactory, somesthetic (vibratory), auditory, and temperature stimuli. None of these was tested systematically in this study, but movement and/or vocalizations from the littermates in the nest appeared to play an important role in the orienting behavior of some subjects on some test trials. This was particularly evident during the latter days of testing when some subjects showed little inclination to move toward the nest until movement in the nest apparently incited their return. The presence of littermates is apparently not a sufficient condition by itself for the

return of baby rabbits to the nest, however, as evidenced by the poor performance of the group placed in a clean nest box on day six.

A final factor which influenced the results of this experiment was the amount of experience of the subjects in the testing situation (i.e., the effect of repeated testing). An analysis of the data on day four in terms of the number of days of testing reveals that 64% of the subjects tested for two days or more (i.e., beginning testing on days one or two) returned to the nest on trial 2; whereas only 24% of the subjects tested for one day or less (i.e., those starting testing on days three or four) returned to the nest. This effect was not evident on any other day, but it is not surprising that such an effect would show up most clearly at an age at which the behavior of many subjects changes suddenly.

The third stage lasted from the sixth or seventh day of age until roughly the end of the second week of life, the age at which many animals began jumping out of the nest box. During this period the young rabbits showed progressively less interest in returning to the nest region when moved, although they were perfectly capable of returning to the nest from any point in the nest box if prodded or frightened.

In general, the demonstration of a stage of development during which infant rabbits show a preferential return to the nest after being moved is quite consistent with the results reported by Rosenblatt, et al. (1962) for kittens and Turkewitz (1967) for rat pups. This stage is both pre-

ceded and followed by a period during which there is no preferential return to the nest region.

Experiment 2: Physical Cliff

One apparatus which seems particularly well suited for the study of perceptual and motor development during the early post-natal period is the physical cliff. Efficient performance in this situation requires the coordination of certain minimal motor abilities with the perception of the somesthetic, kinesthetic, and perhaps auditory (echo) cues associated with the edge. Schneirla (1965) has emphasized the importance of these proximally based somesthetic and kinesthetic sensory mechanisms for perceptual development during this period; while several authors (e.g., Shinkman, 1962) have stressed the probable dominance of visual cues for the physical cliff performance of a number of species tested at later developmental ages.

Experiment 2 is a study of the development of physical cliff performance in rabbits after neonatal brain damage and of the influence of the sudden onset of vision on the established pattern of performance on the physical cliff.

Method

Subjects. A total of 57 rabbits [Orychtolagus cuniculus (L.)] from 11 litters (four California, seven Dutch-belted) were tested on the physical cliff. Of these, 49 animals had received some type of brain lesion at one day of age as follows: 11 with bilateral hippocampal damage, seven with large bilateral cortical lesions (controls for hippocampal

damaged subjects), 12 with small round unilateral cortical lesions, and nine with small round bilateral cortical lesions. The remaining 18 animals served as unoperated control subjects.

Procedure. Four litters were tested in a staggered longitudinal design with training beginning at 1, 2, 3, or 4 days of age, and three litters were tested cross sectionally at 3, 6, or 9 days of age respectively. An additional four litters were subsequently tested for 2 or 3 days from the first evidence of eye opening (days 9 to 11) by any member of the litter. Each subject was run for four trials per day. A trial was terminated when the animal either backed away from the edge or fell off, in which case the experimenter caught it in his hands approximately one foot below. At the end of four trials the rabbit was returned to the nest.

The physical cliff itself consisted of a 12 x 12 x 17 in. triangular area formed by setting the nest box diagonally across one corner of a large transport cage. Figure 12 shows the testing situation. Since there was a drop from each of the 12 in. sides, the subject was actually faced with a double cliff. The top of the cage was 30 in. above the floor.

Results and Discussion

The performance of most subjects improved rapidly through the sixth day of age, and there were no significant differences between lesion groups (Figure 13). The improvement observed during this period appeared to result

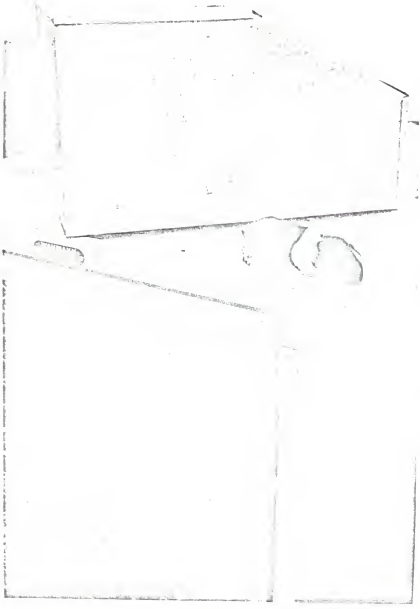


Figure 12. Five day old Dutch-belted rabbit on the physical cliff. California albino female is barely visible in the interior of the transport cage.

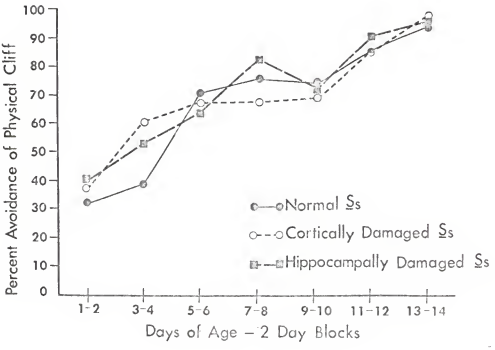


Figure 13. Percentage of trials on which subjects backed away from the edge of the physical cliff as a function of age and lesion group.

primarily from an improvement in motor ability. On days one and two the animals invariably slid off the cliff, often back-peddling with hind and forefeet as they slid, when their heads dropped over the edge. They seemed to lack the ability to shift their center of gravity enough to compensate for the weight of their head extended over the cliff. By day six, however, most subjects froze as soon as their snout and vibrissae extended over the edge. When they fell, it was usually the result of approaching the edge too rapidly.[2]

From day seven through day ten (the day several subjects opened their eyes) there was no further improvement in terms of the percentage of trials on which the animals backed away from the edge. This plateau in the performance curve resulted from the simultaneous development of two opposing trends, the continued improvement of some subjects and an increasing tendency for other subjects to leap from the cliff. This tendency to leap was particularly strong on the last four days before the animal's eyes opened and then ceased abruptly with the advent of vision (Figure 14).

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[2] At about six days of age, the animals first started to use their rear legs effectively to hop and "walk." Prior to that time, the rear legs were extended out to the side and did not support the rabbit's body. Although they could be used to propel the body forward by thrusting to the rear (a sort of jump) the thrust was not well coordinated, and it often resulted in the animal's rolling over (see Experiment 4 below). Most of the locomotion was provided by the forelimbs, and the animal moved inchworm fashion much of the time - extending the forelimbs, drawing up the hindlimbs, and extending the forelimbs again.

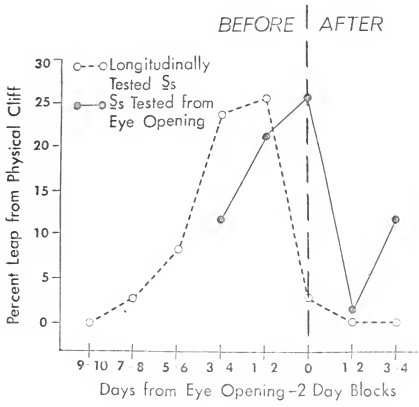

Figure 14. Percentage of trials on which subjects leaped from the physical cliff as a function of the number of days from eye opening and of the testing schedules employed.

In order to test this more thoroughly, four more litters were tested for 2 or 3 days from the first evidence of eye opening by any member of the litter. Since the day of eye opening ranged from 9 to 12 days of age, some subjects in this group started testing just as their eyes began to open, while others were tested for as long as 3 days with their eyes closed.

Somewhat unexpectedly, these subjects also showed a strong tendency to leap from the cliff, a tendency which actually peaked on the day of the animals' eyes opening (Figure 14). Thus, the arrival of vision was not sufficient in itself to prevent the subject from leaping, at least not for the California rabbits. An analysis of the day-of-eye-opening data in terms of race of subjects reveals that this peak is almost entirely due to the behavior of the California subjects. The California albinos leaped on 36% of the trials, whereas the Dutch-belted subjects leaped on only 8% of the trials. On the day prior to eye opening the performance of the two groups was almost identical, with the Californias leaping on 25% and the Dutch-belted subjects on 23% of the trials. This may be due to a difference in visual acuity in the pigmented and nonpigmented rabbits. Lashley (1930) showed that pigmented rats possess considerably greater visual discriminatory capacity than albino rats. Alternatively, or in addition, this difference between races may have resulted from the particular testing situation employed as suggested below. In any event, a certain amount of

prior experience with the cliff also seemed to be necessary for the onset of vision to exert its inhibitory influence on the tendency of these animals to leap from the cliff.

The initial development of the tendency for some subjects to leap from the cliff, however, apparently did not depend on prior experience with the cliff, since both the 9 day old cross-sectional group and the "onset of vision" groups showed a strong tendency to leap on their very first encounter with the physical cliff. This leaves the question of why they leaped in the first place somewhat unsettled, but several bits of evidence suggest that the entire phenomenon of leaping from the cliff may have been an artifact of the testing situation. That is, it may have resulted from the fact that the two California albino females in the breeding colony were too large for the cages in which the Dutch-belted females raised their litters. Therefore, a few days before parturition they were transferred to the large transport cages, one of which doubled as the physical cliff. In essence, then, the baby rabbits may have been leaping toward the female housed below.

When a leap occurred, it was almost invariably from the front of the cage despite the fact that there was a 1/16 in. rim of metal along this edge which should have provided an additional foothold for the subject (see Figure 12). Secondly, one longitudinal group which showed a particularly strong tendency to leap (perhaps because the mother was caged below) was tested on a different transport

cage after regular testing one day. Although six of the nine subjects had leaped on at least one trial during regular testing, not a single animal leaped from the empty transport cage. The next day, when tested on the original physical cliff, four of the six animals leaped on at least one trial (three subjects had been sacrificed). Finally, an analysis of the data in terms of race shows that the Dutch-belted subjects were less likely to leap than the Californias, both before and after the onset of vision. Although this may represent a real difference between races, it seems equally probable, in view of the preceding comments, that the Californias were more likely to leap because their own mother was housed in the cage below.

Experiment 3: Visual Cliff

An apparatus which is similar in some respects to the physical cliff used in the previous experiment, but which eliminates the influence of nonvisual cues on the animal's behavior, is the visual cliff described by Gibson and Walk (1960). The depth perception of a large number of species has been studied on this apparatus (e.g., Walk, 1965), and in general all types of animals discriminate well between the two sides (i.e., avoid the optically deep side) by the age at which they normally begin to move about freely (Walk and Gibson, 1961). In rabbits this would be roughly between 10 and 15 days of age (at or shortly after eye opening).

The following is a study of the development of depth perception in infant pigmented and nonpigmented rabbits with and without neonatal brain damage.

Method

Subjects. Ten albino (California) rabbits from three litters and 25 pigmented (Dutch-belted) rabbits from five litters were tested on the visual cliff between 10 and 14 days of age. Eleven of these were unoperated control subjects. The remainder had received some type of brain lesion at one day of age as follows: ten with bilateral hippocampal lesions, five with bilateral cortical lesions (controls), seven with small round unilateral cortical lesions, and two with small round bilateral cortical lesions. All of these subjects had been tested at least once on the physical cliff of the previous experiment.

Procedure. The visual cliff was 30 x 30 x 24 in. A red and white 2 x 2 in. checkered pattern covered the walls, the underside of the glass on the "shallow" side, and the floor of the "deep" side 16 in. below the glass. A runway elevated 2 1/2 in. above the glass separated the "deep" from the "shallow" side. This runway was covered with a layer of gauze to provide the young rabbits with a foothold. All illumination was provided by a pole lamp containing a single 15 watt bulb placed behind the center of the optically shallow side (see Figure 15).

Five litters (three California, two Dutch-belted) were tested for 2 or 3 days in succession starting on the day

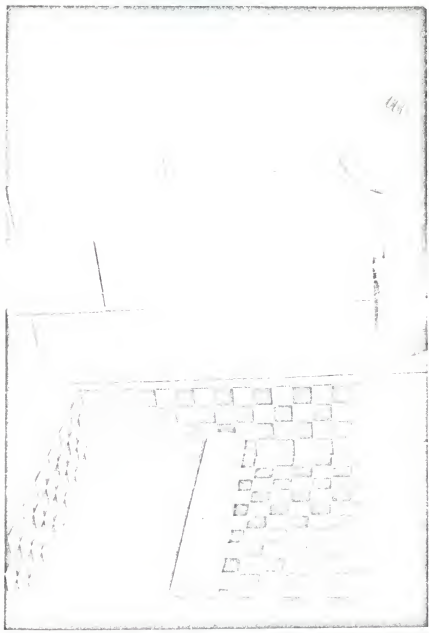

Figure 15. Photograph of visual cliff with gauze covered runway. All illumination is provided by a single 15 watt bulb located above the optically shallow side.

when the first member(s) of the litter opened its eyes. As a result, some animals started testing with their eyes fully open, some with one or both eyes partially open, and some with both eyes closed. The four animals which started testing with their eyes closed provided a convenient control for any possible systematic bias in the testing situation. Three additional litters were tested cross sectionally 1 day (two litters) and 3 days (one litter) after their eyes opened.

The rabbits were placed one at a time in the center of the gauze covered runway[3] and allowed a maximum latency of 300 sec. (5 min.) to descend. If no descent occurred within that time, the maximum latency was recorded and the trial terminated. If a subject descended (or fell) to either side within the 5 min. period, the latency and side of descent were recorded and the trial terminated 15 sec. later. During the 15 second post-descent period the behavior of the subject (particularly direction of movement) was observed. No distinction was made between falls and what appeared to be deliberate descents. Occasionally, one or both of the animal's rear legs would slip off the runway onto the glass on one side or the other. This seemed to have no effect on the

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[3]One group of pilot subjects was tested on the visual cliff with the original uncovered slate runway. These animals performed very poorly, slipping off the runway about equally often on each side whenever they looked over the edge. When the runway was covered with gauze, their performance improved dramatically.

animal's subsequent choice behavior and was not scored as a descent unless the animal fell all the way off the runway (i.e., all four feet on the glass).

Results and Discussion

The day by day results for all subjects are presented in Table 5 and Figure 16. Of the four subjects which started testing on the day before their eyes opened, two fell onto the deep side and two did not descend within the 300 sec. of the trial. Thus, if there was any systematic bias in the experimental situation (e.g., unstable or tilted runway) it would seem to favor deep side descents.

On the day of eye opening, the performance of pigmented rabbits was superior to that of nonpigmented rabbits. Seventy-three percent of the Dutch-belted subjects, but only 50 % of the California subjects, descended to the shallow side. By the next day, however, this difference had disappeared. There were no lesion effects in terms of descents on any day.

This difference between races is analogous to the results obtained by Walk and Gibson (1961) and Routtenberg and Glickman (1964) with rats. Both studies showed that young (26-31 day old) hooded rats descend initially to the shallow side much more often than albino rats of the same age. These visual cliff results lend some credence to the idea that the observed differences in behavior on the physical cliff of the previous study between pigmented and nonpigmented rabbits on the day of eye opening result from a difference

TABLE 5

Performance of Infant Rabbits on Visual Cliff from Day before until Three Days after Eye Opening

			California	Dutch-belted	Total
		N	2	2	4
Day -1	%descent	Deep Side	0%	100%	50%
		Shallow Side	0%	0%	0%
		No descent	100%	0%	50%
		N	8	11	19
Day 0 (Eyes open)	%descent	Deep Side	50%	18%	32%
		Shallow Side	50%	73%	63%
		No descent	0%	9%	5%
		N	10	16	26
Day 1	%descent	Deep Side	10%	19%	15%
		Shallow Side	80%	75%	77%
		No descent	10%	6%	8%
		N	4	4	8
Day 2	%descent	Deep Side	25%	25%	25%
		Shallow Side	75%	75%	75%
		No descent	0%	0%	0%
		N	0	7	7
Day 3	%descent	Deep Side	-	0%	0%
		Shallow Side	-	100%	100%
		No descent	-	0%	0%

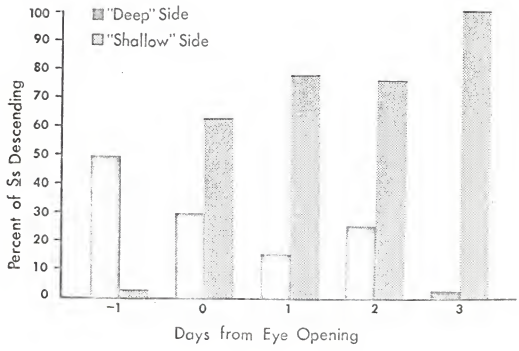

Figure 16. Percentage of subjects descending to the deep or to the shallow side of the visual cliff as a function of the number of days from eye opening. Since some subjects did not descend from the runway, the percentages may not total 100%.

in visual acuity. However, other sections of Walk and Gibson's (1961) study (besides the visual cliff performance) suggested that the ability to perceive depth was similar in the two strains of rats. Also, Schiffman (1970) reported that both pigmented and albino rabbits six weeks of age have efficient depth perception, as demonstrated by the fact that both avoid the optically deep side of a visual cliff equally well when placed initially on the shallow side. In contrast, when the rabbits were placed on the deep side, only the pigmented rabbits showed a strong tendency to move to the shallow side. Routtenberg and Glickman (1964) obtained similar results with adult albino and hooded rats and suggested that both visual acuity and emotionality play a part in a subject's cliff behavior. Thus, differences in emotionality (as reflected in fecal bolus counts and open-field behavior in Routtenberg and Glickman's study) between strains may largely account for the strain differences observed in perceptual tasks such as the visual cliff.

On the other hand, observations of the subject's behavior for 15 seconds after a descent suggested that both pigmented and nonpigmented rabbits in this experiment were "fearful" after a fall onto the deep side. No subject of either race ever really descended onto the deep side as they often did onto the shallow side, but when a subject fell onto the deep side it usually froze with legs outstretched. If any movement occurred it was usually in the direction of the runway (one Dutch-belted subject immediately leaped back

onto the runway after falling onto the deep side and then descended to the shallow side 30 sec. later), and there were no obvious differences between races. However, it is conceivable that if accurate measurements of time, direction, or length of locomotion had been recorded, results similar to those of Schiffman (1970) and Routtenberg and Glickman (1964) might have been obtained. Most subjects also froze momentarily after a fall onto the shallow side but soon started to move around; whereas locomotion and exploration followed almost immedaitely after a deliberate descent to the shallow side.

The sudden improvement in the performance of the California subjects on the day after eye opening may be attributable to any of a number of factors such as: (1) a further degree of eye opening (the fact that several subjects started training with one or both eyes only partially open might have had a particularly adverse effect on the California subjects in view of the evidence that albino animals may have poorer visual capacities to start with); (2) physical maturation of the visual system for an additional day beyond eye opening; (3) nonspecific visual experience between the two days of testing; (4) specific experience with the visual cliff on the previous day. This last factor might seem like a particularly important one considering the apparent importance of specific experience with the testing situation for the performance of the California subjects on the physical cliff after the onset of vision. However, a com-

parison of the performance of those California subjects tested sequentially on the two days with that of the three California subjects tested for the first time on the day after eye opening reveals that: (a) 87% of the sequential group descended on the shallow side while 13% descended onto the deep side (b) two of the three (66%) subjects in the cross-sectional group descended on the shallow side and the other subject did not descend. Although this trend in the expected direction, it would be dangerous to conclude that specific experience is the prime factor, or even an important factor, considering the small number of subjects in the cross-sectional group. Furthermore, in a longitudinal study of infant albino rabbits on the visual cliff from eye opening until 30 days of age, Walk (1966) found a very gradual improvement in performance over days. His subjects required more than 10 days to reach the level of performance attained by the subjects in this study on the first day after eye opening.

Several procedural differences between Walk's (1966) study and the present experiment might account for the apparently discrepant results. The animals in this study had all received considerable handling prior to testing. They had been weighed daily and each sujbect had been tested at least once in one or more of the other three behavioral situations reported here (see Appendix B). Lore and Sawatski (1969) showed that infant hooded rats which received daily handling were superior to unhandled subjects

when tested on the visual cliff shortly after eye opening.

Another difference between the two studies is the fact that the illumination of the two sides of the cliff was not equated in the present experiment (Figure 15). It is possible that the dimmer illumination of the optically deep side of the cliff may have enhanced the illusion of depth and led to the rapid improvement in this study.

Finally, Walk tested his subjects longitudinally for 30 days. As a result, some of his subjects may have learned that a fall or descent onto the deep side has no punishing consequences, thereby reducing the tendency to avoid the deep side and flattening the day to day performance curve. On the contrary, the subjects in the present sutdy had been tested at least once on the physical cliff. This experience may have taught some of the subjects that a fall from the edge has mildly aversive properties. In addition, Walk (1966) reports, "... the rabbit has a tendency to stop responding, far more so than the kitten, [so] the animals were usually given two trials a day and frequently tested on alternate days, or even less often " (p. 90). Such a tendency for some subjects not to descend would also decrease the percentage descending to the shallow side and flatten the performance curve.

In conclusion, both pigmented and nonpigmented rabbits are capable of efficient visual cliff performance shortly after their eyes open if the experimental situation is favorable, but pigmented rabbits are superior to nonpigment-

ed rabbits in depth avoidance for any of a number of possible reasons. Furthermore, infant rabbits are capable of normal depth avoidance even after neonatal brain damage (of the type induced in this study) and the resultant morphological alterations observed.

Experiment 4: Orienting-Jump Response

In an ontogenetic study of New Zealand White rabbits, Fox and Apelbaum (1969) described the development of a stereotyped defensive behavior pattern which they called the orienting-jump (O-J) response. They showed that this complex behavior pattern consists of a number of response components which appear at different ages. Earlier appearing reflex-like (unvarying and regularly elicited) components are either supplanted by or become integrated with later appearing and often more variable components as the animal matures.

The present study was designed to examine the effects of neonatal brain damage on the appearance and elaboration of the components of the O-J response in two races of rabbits. Since specific components of the O-J response appear at different times during the first few weeks of life, concurrently with the morphological (and presumably physiological) adjustments to infant neurological damage noted previously, it was thought that this might be a particularly sensitive measure of the effects of these processes on motor function and development.

Method

Subjects. Twenty-two subjects from three litters (one Dutch belted, two California) were tested longitudinally. The

Dutch-belted litter was tested from the first through the fifth day of life, and the two California litters were studied from the second through the sixteenth and from the third through the twenty-fourth day of life respectively. An additional 42 subjects from nine litters (six Dutch-belted, three California) were studied cross sectionally, being tested at 6 (two litters), 8 (one), 9 (one), 10 (one), 12 (one), 18 (two), or 24 (one) days of age. Of the 64 total subjects, 27 were unoperated control animals. The remaining 37 subjects had received some type of infant brain damage as follows: ten with bilateral hippocampal damage, six with bilateral cortical damage (controls), 12 with small round unilateral cortical lesions, and nine with small round bilateral cortical lesions. Five unoperated Dutch-belted adults (one male, four females) were also tested.

Procedure. Testing occurred at approximately the same time every day after the animal was weighed. The testing procedure was basically the same as the one reported by Fox and Apelbaum (1969). The subjects were placed individually on a 21 x 29 in. steel utility table with a 1 1/2 in. raised rim all the way around the edge. Punctate stimulation was applied twice to each thigh at 10-15 sec. intervals with a 20 gauge blunt stainless steel hypodermic needle. Response components were scored as being either present or absent in each subject on any of the four trials. The response components are described under results and discussion.

Results and Discussion

The results obtained were roughly the same as those reported by Fox and Apelbaum (1969) with a few exceptions as noted below. There were no differences between the two races except that the Dutch-belted subjects seemed somewhat more aggressive. No lesion effects were obtained. The overall results for all subjects combined are presented in Table 6 after the manner of Fox and Apelbaum (1969).

Directed and nondirected escape. Immediately after the oriented jump (or forward or double jump) almost every subject attempted to escape from the probe. During the first week of life the direction of escape showed little or no relation to the location of stimulation (i.e., no orientation) and the subject often moved in circles. This component, which is termed nondirected escape, was gradually replaced by directed escape (i.e., obvious relation to location of probe and experimenter). Since more than one stimulation was delivered each day, these categories are not mutually exclusive. Some subjects showed directed escape to some stimuli and nondirected escape to others. Nondirected escape was entirely replaced by directed escape when the subjects' eyes opened on days nine to twelve.

Oriented jump. The oriented jump, the major component of the O-J response, is described by Fox and Apelbaum as a jump and turn towards the side of stimulation, bringing the subject face to face with the probe (i.e., 180° turn). In the present study, this component showed a more gradual on-

TABLE 6

Age of Occurrence of Components of O-J Response (Days)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	24
Nondirected Escape	+	+	+	+	+	-	+	-	+	-	-	-	.	.	.	.	-	-
Directed Escape	.	.	.	-	-	+	-	+	+	-	+	+	+	+	+	+	+	+
Forward Jump	+	+	+	+	+	+	+	+	+	+	-	-	.	.	.	.	-	-
Oriented Jump	.	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rolling Over	+	+	+	-	-	+	-	-	.	.	.	.	.	.	.	.	.	.
Hind-Limb Thrust	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Double Jump	.	-	.	-	-	-	-	-	+	+	+	-	+	-	.	.	-	.
Fore-limb Stab	.	.	.	.	-	-	-	-	-	-	+	-	+	+	-	-	-	-
Bite Attack	.	.	-	-	-	+	-	-	-	-	-	-	+	-	.	.	.	-
Vocalization	+	-	-	-	-	+	-	-	+	-	-	-	-	-	.	.	.	.
Popcorn effect	.	.	-	-	+	+	+	+	+	+	+	+	+	-	.	.	.	.

Plus indicates component present in more than 50% of subjects.

Dash indicates component present in 1% - 50% of subjects.

Period indicates total absence of component.

set and was more variable in degree than they reported. A full 180° turn was first seen at 6 days of age in one litter and was not seen in a large number of subjects until 8 days of age. Prior to that time the orientation was only partial (i.e., 30° or 45° turn). For purposes of tabulation, only responses with at least a 45° turn were considered oriented jumps. After 8 days of age, most subjects showed both complete and incomplete responses on different trials of the same day. One reason for this was that the development of the oriented jump sometimes occurred at different rates for the two sides of the body. That is, some subjects consistently showed a stronger orientation to punctate stimulation of one side of the body than the other. Although this tendency was often consistent within subjects, it seemed to be unrelated to side or degree of neurological damage; stronger orientation to stimulation of the right side predominated over the reverse situation only slightly.

In agreement with Fox and Apelbaum's data, the oriented jump was much harder to elicit after 12 days of age (i.e., about the time of eye opening), particularly in the cross-sectionally tested subjects and particularly if the subject was alert and exploring the table. However, when an oriented jump was elicited in these subjects it was almost always complete. Intensity of stimulation did not seem to be important; a response was elicited equally often with either a brisk jab or a light tap. In the oldest cross-sectional groups (18 and 24 days old) the first reaction of

most subjects was to crouch and freeze. After two or three repeated stimuli they showed very strong directed escape. The oriented jump was elicited only when the subject was cornered and unable to escape;[4] then it was usually accompanied by a fore-limb stab and/or bite attack (see below). The longitudinally tested subjects of the same age were somewhat less likely to freeze, but directed escape was still the predominant component, and the oriented jump was usually elicited only after two or three stimulations. When the oriented jump occurred, it was less likely to be accompanied by a forelimb stab or a bite attack than in the cross-sectional groups.

The oriented jump was extremely difficult to elicit from the adults. Two of the females were very aggressive, and any attempt to approach them with the probe was rebuffed with a combined fore-limb stab and a bite attack. The other two females and the male were quite docile and punctate stimulation with the probe elicited only directed escape followed by crouching and huddling in a corner.

Forward jump and double jump. During the first 10 days, a forward jump (i.e., no orientation) was often elicited, especially on the first stimulation. As the degree of orientation increased in most subjects over days, the number of

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[4]These subjects were tested on the floor because the 1 1/2 in. rim around the utility table was not sufficient to prevent them from leaping or falling off.

forward jumps correspondingly decreased. At the same time, some subjects showed a tendency to execute two rapid 180° turns to a single stimulation. This double jump was particularly evident from day 9-13 in association with the "popcorn" effect (see below).

Hind-limb thrust and rolling over. In executing the oriented jump (or forward or double jump), the animal would simultaneously extend or thrust both hind legs. During the first few days of life this thrust was not well coordinated, and the animal would often roll over onto its side.

Fore-limb stab and bite attack. The oriented jump, which placed the subject opposite the probe, was sometimes followed by a bilateral strike at the probe with the forepaws. This was most likely to occur one or two days after the animal opened its eyes. A bite at the probe also occurred on some trials. This component was more easily elicited by a light tap on the shoulder or head than by punctate stimulation of the thigh. Only bite attacks elicited during regular testing (i.e., after probe on the thigh) are included in Table 6.

Vocalization. This extremely variable component (both between and within subjects) occurred more often in the younger age groups than the older ones, and not at all after 14 days of age. However, vocalizations were elicited from two of the adults in association with the bite attack and fore-limb stab.

The popcorn effect. At a certain age, arousal of one

subject by stimulation causes a sort of chain reaction, and the entire litter begins to respond. Fox and Apelbaum (1969) assigned this phenomenon the descriptive title of "the 'popcorn' effect." The development of this component very closely paralleled the development of the oriented jump in this experiment. One litter showed "popcorn" as early as 2 days of age, and two more litters began to respond on days three and four respectively. It was observed in all litters from 5-11 days of age, and disappeared completely at day 15. This correlated very closely with the decrease in ease of eliciting the oriented jump after 12 days of age. Similarly, Volochov (cited in Molakhovskaia, 1961) reported a decrease in specialized skin reflexes to mechanical stimulation after the second week of life which disappeared altogether at 16 days of age.

In contrast, Fox and Apelbaum (1969) report both a later onset and a later offset of the "popcorn" effect, with all of their litters responding from 8-18 days of age. The reason for this discrepancy between the results of the two studies is not immediately evident. It may be due to the different races used, but this seems unlikely since both the Dutch-belted and the California albino litters of the present study were responding by day five. Moreover, both races ceased responding by day 15. Considering the close genetic relationship between the New Zealand White rabbits used in Fox's and Apelbaum's (1969) study and the California albinos used in this study (Ross, et al., 1963), it does not seem

likely that the obtained differences in ontogeny of the "popcorn" effect are due to race differences.

Hind-limb appel. This component is not listed in Table 6 because none of the subjects showed it during regular testing. One of the 18 day old and two of the 24 day old subjects, however, showed it when they were placed back in their cage. One hind foot was brought down sharply against the cage floor producing a sharp thump or appel. This was also seen in the two aggressive adults along with vocalization and attack.

GENERAL DISCUSSION

Considering the fact that an increase in perceptual and motor experience leads to a corresponding increase in cortical thickness (Diamond, Krech, and Rosenzweig, 1964) and length of cerebrum (Altman, Wallace, Anderson, and Das, 1968) in rats, it is somewhat surprising that the opposite effect was not obtained in the present study. That is, it is somewhat surprising that the rather dramatic morphological changes resulting from early brain damage in rabbits did not produce any measurable effect on perceptual-motor development in this study. It might be argued that the perceptual-motor capacities tapped by the four tasks used in this study are subcortically mediated, at least during the early postnatal period, and that the lesion was restricted to a very small patch of posterolateral neocortex bordering visual and somesthetic areas (Rose and Woolsey, 1949). However, the observed reduction in hemisphere growth which resulted from this lesion was not restricted to neocortex, but extended into diencephalic and midbrain regions as well. In any event, it appears that the brain damaged animals performed well on these perceptual-motor tasks in spite of the observed morphological changes rather than because of them. Therefore, if we are to find a morphological basis for functional recovery (or sparing) after early brain lesions, we shall

almost certainly have to look beyond gross morphological changes of the type reported both in this study and in many of the sensory-motor enrichment studies (e.g., Altman, et al. 1968).

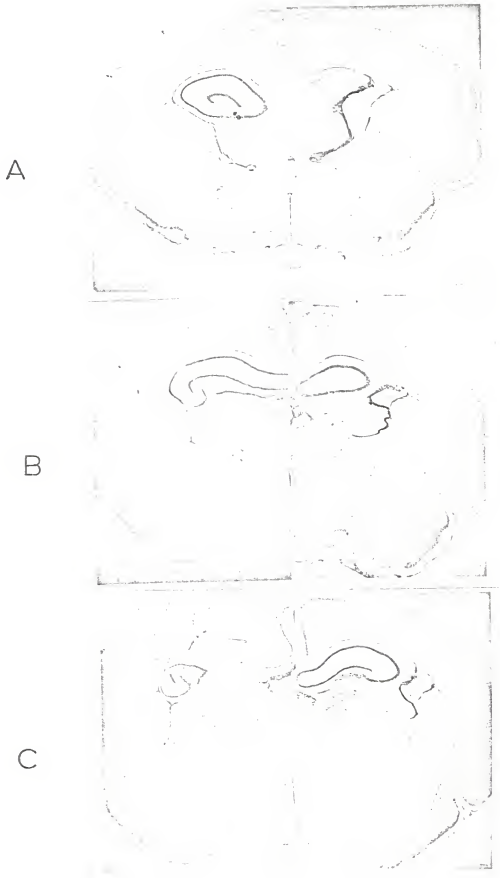
One possibility might be increased or prolonged neurogenesis resulting from the early lesion. Such an effect might conceivably take place in response to the removal of some inhibitory agent on subsequent neurogenesis (purely hypothetical at present) produced by exisitng differentiated or differentiating neurons. One study which lends credence to this suggestion is a recent report by Altman, Anderson, and Wright (1969). In that study, a single 200 r dose of X-ray to the heads of infant rats produced necrosis of cells in the external granular layer of the cerebellum which led to a drastic reduction in width of this layer by 48 hours after irradiation. By the third day after irradiation this layer began to increase in width and by the fourth day it appeared structurally normal. Thus, the external granular layer in the cerebellum of the infant rat seems to have considerable restorative capacity. It seems likely that such a restorative process would be most pronounced in those structures which are still undergoing neurogenesis at the time of injury or in which neurogenesis has recently stopped and in which there is still a large population of incompletely differentiated neurons.

In normal hippocampus, production of pyramidal cells for fields CA1 and CA3 continues up to the day of birth in

mice (Angevine, 1965) and granule cell production for the dentate gyrus continues for some time after birth both in mice (Angevine, 1965) and in rats (Altman and Das, 1965), perhaps even into adulthood (Altman, 1969). This prolonged postnatal neurogenesis of dentate granule cells arises partially from regionally proliferating undifferentiated cells located within stratum granulosum itself, a considerable distance from the ependymal and subependymal proliferative zones. Similarly, in electron microscopic studies Caley and Maxwell (1968a,b) have identified undifferentiated ("indifferent") cells in the cortex of young rats which subsequently differentiate into neuroblasts and spongioblasts (precursors of glia cells).

In view of this autoradiographic and electron microscopic evidence of a postnatally active population of proliferative cells some distance removed from the germinal region lining the ventricles, it is tempting to speculate on the possibility that the abnormal growth of a structure which sometimes occurs after neonatal injury might result from prolonged or renewed neurogenesis in response to the injury. One example of such abnormal development was shown in Figures 8 and 9 of the present study. Another example of abnormal hippocampal development which apparently resulted from neonatal surgical insult is pictured in Figure 17. This animal received bilateral hippocampal lesions at 1 day of age as part of the series of behavioral studies reported in Chapter 2. Only a tiny remnant of the hippocampus

Figure 17. Photographic montage of abnormal rostral development of hippocampus in 5 week old rabbit. Right half of picture shows hemisphere of 3 1/2 week old normal rabbit for comparison. The 3 1/2 week old brain was chosen for this purpose because it more closely matches the 5 week old damaged brain in terms of size and structural development than does the brain of a 5 week old normal animal.


A
B
C

remains at the level of maximal hippocampal destruction (Figure 17c), but from this point it burgeons out into an abnormally large structure anteriorly which continues rostrally in the lateral ventricle to levels which normally contain no hippocampal tissue.

Of course, such instances of abnormal growth after neonatal injury may result entirely from growth of exisiting neurons and their processes and from glial proliferation. The abnormal morphological appearance might result from a scrambling of some cellular elements at the time of the insult and the subsequent reestablishment of semi-normal histological relationships between them during growth. In this regard, it is of interest that isocortical and hippocampal neurons grown in a liquid culture medium tend to form aggregates whose cellular arrangements very closely resemble the histological appearance of the normal parent structure (DeLong, in press).

Finally, one other way in which the brain might compensate for early injury would be through axonal sprouting of any remaining intact fibers from the damaged area or of fibers from anatomically distinct but functionally related areas. The fact that axonal sprouting occurs in many parts of the central nervous system of adult mammals after partial deafferentation has now been well established (for a review see Raisman, 1969), and it seems reasonable to expect that such a process might be even more pronounced in the rapidly developing brain of the neonate.

At any rate, it is clear that the question of whether or not there is a morphological basis for recovery of function after early brain injury cannot be answered with certainty on the basis of present day knowledge. Recent developments in a number of different areas of brain research have provided several intriguing leads, some of which have been mentioned above. Hopefully, by following these leads both at the structural and the ultrastructural level, a partial answer may soon be at hand, although the final picture will undoubtedly be a complex one.

APPENDICES

APPENDIX A

Autoradiographic Procedure For Tritiated Thymidine Labeled Tissue

Perfusion and Fixation

Intracardial perfusion with normal saline followed by 10% formalin. Fix in Carnoy's fluid made as follows:

absolute alcohol----------60ml.

chloroform----------30ml.

glacial acetic acid------ 10ml.

Embedding and Sectioning

Embed in paraffin using standard procedures, section at 6-8 microns (tritium is a weak beta emitter), and place on frosted-end slides coated with gelatin. Frosted-end slides are used so you can tell by feel which side tissue is on. Albumen causes excessive background, so gelatin is always used.

Preparing Slides for Tissue

Clean slides in acid-bichromate cleaning solution (even commercially available "pre-cleaned" slides are usually too dirty), rinse in running water several hours and then in distilled water in staining dish for 10 min. or more. When dry, dip slides in following solution at room temperature:

gelatin---------- 5.0 gm.

chrom-alum---------- 0.5 gm.

distilled water, to make --1,000 ml.

pinch of thymol

Filter immediately before use. Don't store longer than about 48 hrs., even in a refrigerator.

Deparaffinization

Deparaffinize sections in clean xylene. Take slides from xylene through alcohol to distilled water to rehydrate tissue before coating with emulsion. Warm slides in distilled water at 45°C. This lessens sudden cooling of emulsion, letting it spread on slide more evenly.

Coating Slides with Liquid Emulsion

1. Remove emulsion from refrigerator and let stand in its light-tight container until the following are done.
    a) Make sure dark room is clean to avoid contaminating emulsion and ruining autoradiograph with previously spilled, dried chemicals.
    b) Check humidity of dark room. Ideal humidity of around 45% prevents background caused by too rapid drying of emulsion.
    c) Get the following things ready: Water bath at 45°C, slide warmer at 45°C, 100ml. beaker, small vessel for dipping slides, test tube rack for draining coated slides, slide boxes, electrical tape, small packets of silica-gel or Drierite wrapped in gauze.
    d) Turn off lights except for single 15 watt bulb behind Kodak Wratten #2(red) Safelight filter at least three feet away. Plug in all

electrical appliances before opening emulsion.

2. Remove plastic bottle of emulsion from its light-tight container and place in water bath. When emulsion has liquefied (15-30 min.), stir very gently with a clean glass rod. Slowly pour about half of the emulsion into the 100 ml. beaker and allow to stand in the water bath for another hour to let air bubbles come to the surface. At the same time, fill small vessel used for dipping slides. Thereafter, refill the vessel as needed from the beaker. About 600 slides can be coated with a full four ounce bottle of emulsion.
3. Take two of the slides from the warm water, place them back-to-back, tissue side out, and shake off excess water. Then holding the slides by their frosted ends, dip into the emulsion. Withdraw smoothly, being careful not to drip excess emulsion back into container.
4. Separate the slides and permit them to drain for a moment onto a gauze pad. Wipe excess emulsion from back and end of slide, and stand slide upright against rack to allow emulsion to gel. Laying slides in horizontal position produces a somewhat more even emulsion layer but also increases the danger of airborne dirt or chemicals settling on the emulsion.
5. Place the dried slides in black plastic boxes containing one of the packets of silica-gel or Drierite.

When all of the coated slides have been placed in boxes, seal each box with black plastic electrical tape. Store boxes on end in refrigerator at 5°C so that slides are exposed in a horizontal position, emulsion side down. Exposure time varies with many factors, but about 3 weeks should be sufficient for tissue with 4 μCi/gm. dose of tritiated thymidine. Best exposure time for particular conditions employed can, and should, be determined empirically.

Developing

1. Fill three glass staining dishes with appropriate solutions (see below), and put in tray of water at 17-19°C. If tap water is too warm to be adjusted to this temperature, add sufficient ice to maintain a constant temperature. Add more ice as needed.
2. Turn out lights except for safelight. Remove slides from their exposure boxes and place in glass slide holder. Process in following solutions:
    a) Kodak D-19 Developer
    b) Kodak SB-5a Stop Bath made as follows:
        1) Acetic acid (28%)----------192 ml.
        2) Distilled water ------- 1,500 ml.
        3) Anhydrous sodium sulfate --135 gm.
        4) Mix above, then add water to make 3 liters
    c) Kodak Acid Fixer (Hypo)
3. Developing time should be empirically determined by

taking a batch of slides and running a couple at a time for varying lengths of time. Then select best time for particular stain and microscopic viewing conditions employed. However, the following is a rough guide for Kodak NTB-2 emulsion.

a) Develop for 5-7 minutes with constant agitation. Choose a time that gives clear labels without too much background.

b) Put directly in stop bath for 15 seconds.

c) Fix for 2 min. with constant agitation. Then turn on light to see if emulsion is clear (being sure that exposure chamber has been closed first). Fix for about 4 min. more, or a little longer if emulsion was not clear.

d) Rinse in water (running if possible) at same temperature as processing solutions for about an hour.

Staining (Mayer's hematoxylin)

1. Rinse slides in water.
2. Stain in Mayer's hematoxylin for 5-7 minutes.
3. Dip in distilled water. Do not "blue" the stain with water. Best staining results from an unconventional red-orange hematoxylin, against which silver grains are visible.
4. Quick rinses in 95% alcohol, absolute alcohol, 1/2 absolute alcohol and 1/2 xylol, xylol. Mount coverslips from xylol with Permount.

Fixatives

Safest fixatives are absolute methanol or Carnoy's fluid. Salts of mercury and lead cause reaction with emulsion so solutions containing them (e.g., Zenker's fluid) should not be used. Formalin is commonly used but may cause desensitization of the emulsion. Glutaraldehyde may have similar effect. Fixatives with picric acid (e.g., Bouin's solution) can be used but the resultant yellow coloration should be removed with ammoniacal alcohol or lithium carbonate while section is being dewaxed. Chances of chemography from any fixative can be reduced by prolonged washing of tissue in water.

Stains

Many stains give satisfactory results with autoradiographs of brain tissue. The best results are obtained from a light stain against which the developed silver grains can be clearly seen. Eosin, thionin, and acid fuchsin stain the emulsion and should therefore be used before emulsion application if desired. Cresyl violet, gallocyanin, toluidine blue, and hematoxylin do not stain the emulsion, but hematoxylin is removed during developing and must therefore be post-stained. If hematoxylin and eosin staining is desired, eosin staining can be done before emulsion application and hematoxylin staining after photographic processing. If gallocyanin is desired, do not heat the stain as recommended for routine use. Gallocyanin is taken up slowly and heating hastens the staining process, but it also causes the emulsion to peel off the slide.

Controls

Several controls are necessary with each experiment in order to validly interpret the autoradiographs obtained. First, each slide should be used as its own control by observing the background of developed silver granules occurring over natural or artifactual tissue spaces (e.g., ventricles, tears) and in areas of the slide relatively remote from the tissue. This background should then be subtracted from the count observed over presumably labeled tissue. Also, specific controls are needed to exclude spurious counts or loss of counts due to some reaction of emulsion with the tissue (chemography). The occurrence of false positives can be checked by including one slide in each box with tissue which is known to be nonradioactive but which has been treated the same as the experimental tissue in all other respects. False negatives can be checked by including one slide which has been exposed to light or to some other source of external radiation and checking for fading over the tissue. If the emulsion is used for more than one batch, a coated slide without tissue should be included from time to time to check the fog level of the emulsion. Discard emulsion if the fog level is too high.

APPENDIX B

Experimental History for all Ss Including Age at Beginning of Testing for Each Testing Situation

S	Lesion Type	Tritiated Thymidine	Age at ^{3}H-T Injection	Nest Return	Physical Cliff	Visual Cliff	O-J Responses
1-2	UC						
1-4	BC						
1-6	UC						
1-7	UC						
1-8	UC						
1-9	BC						
1-10	BC						
2-4	UC	1 µCi/gm	5wks				
2-6	N	1 µCi/gm	5 wks				
2-7	N						
3-1	BC			L 3 Days X	3 Days		L 2 Days
3-2	BC			L " X	"		L "
3-3	BC						X "
3-4	BC	1 µCi/gm	3 wks	L 3 Days X	3 Days		L "
3-5	UC			L " X	"		L "
3-6	UC	1 µCi/gm	3 wks	L " X	"		L "
3-7	UC			L " X	"		L "
3-8	N	1 µCi/gm	3 wks	L " X	"		L "
3-9	N			L " X	"		L "
4-1	N	1 µCi/gm	1 Day	L 1 Day L	1 Day		L 1 Day
4-2	N	1 µCi/gm	1 Day	1 " L	"		L "
4-3	BC	1 µCi/gm	1 Day	L " L	"		L "
4-4	BC	1 µCi/gm	1 Day	L " L	"		L "

APPENDIX B
continued

S	Lesion Type	Tritiated Thymidine	Age at ^{3}H-T Injection	Nest Return	Physical Cliff	Visual Cliff	O-J Responses
5-1	N(BC)						X 6 Days
5-2	N	2 µCi/gm	4 Days				
5-3	N						X 6 Days
5-4	N						X "
5-6	N						X "
5-7	N						X "
5-9	N						X "
5-10	N(UC)						X "
5-11	N						X "
5-12	N						X "
6-1	N(UC)			L 1 Day L	2 Days X	14 Days	X 10 Days
6-2	C			L " L	" X	"	X "
6-3	HPC			L " L	" X	"	X "
6-4	C			L " L	" X	"	X "
6-5	C			L " L	" X	"	X "
6-6	HPC			L " L	" X	"	X "
6-7	HPC			L " L	" X	"	X "
6-8	N(UC)			L " L	" X	"	X "
7-1	C			X	4 Days		
7-2	HPC			L 4 Days L	" X	12 Days	X 12 Days
7-3	HPC			L " L	" X	"	X "
7-4	HPC			L " L	" X	"	X "
7-6	N			L "			
8-1	N			XL	10 Days XL	11 Days	X 24 Days
8-2	N			XL	" XL	"	X "
8-3	N			XL	" XL	"	X "
8-4	N			XL	" XL	"	X "
8-5	N			XL	" XL	"	X "
8-6	N			XL	" XL	"	X "

APPENDIX B
continued

S	Lesion Type	Titriated Thymidine	Age at ^{3}H-T Injection	Nest Return	Physical Cliff	Visual Cliff	O-J Responses
9-1	UC				XL 11 Days	X 12 Days	X 18 Days
9-2	UC				XL "	X "	X "
10-1	UC			L 5 Days	XL 10 Days	XL 9 Days	X 9 Days
10-2	BC			L "	XL "	XL "	X "
11-1	C			L 2 Days	XL 9 Days		X 8 Days
11-2	HPC			L "	XL "		
11-3	C			L "	XL "	XL 10 Days	X 8 Days
11-4	HPC			L "	XL "	XL "	X "
11-5	HPC			L "	XL "	XL "	X "
11-6	C			L "	XL "		X "
11-7	HPC			L "	XL "	XL 10 Days	X "
11-8	N			L "	XL "		
11-9	N			L "	XL "		X 8 Days
12-1	HPC			L 2 Days	X 9 Days	XL 10 Days	X 18 Days
12-2	HPC			L "	X "	XL "	X "
12-3	C			L "	X "	XL "	X "
12-4	N			L "	X "	XL "	X "
12-5	N			L "	X "	XL "	X "
13-1	UC	4 μCi/gm	1 Day				
13-2	UC	"	2 Days				
13-3	UC	"	4 Days				
13-4	UC	"	8 Days		X 6 Days		X 6 Days
13-5	N	"	2 Days				
13-6	N	"	4 Days				
13-7	N	"	8 Days		X 6 Days		X 6 Days

APPENDIX B
continued

S	Lesion Type	Tritiated Thymidine	Age at ^{3}H-T Injection	Nest Return	Physical Cliff	Visual Cliff	O-J Responses
14-1	UVNC			L 3 Days	L 3 Days	XL 11 Days	L 3 Days
14-2	UHNC			L "	L "	XL "	L "
14-3	URNC			L "	L "	XL "	L "
14-4	UC			L "	L "		L "
14-5	UC			L "	L "	XL 11 Days	L "
14-6	BC			L "	L "		L "
14-7	BC			L "	L "	XL 11 Days	L "
14-8	N			L "	L "		L "
14-9	N			L "	L "	XL 11 Days	L "

Legend: UC Unilateral Circular Cortical Lesion
BC Bilateral Circular Cortical Lesion
UVNC Unilateral vertical needle cuts (())
UHNC Unilateral horizontal needle cuts (⊃)
URNC Unilateral round needle cuts (o)
HPC Bilateral Hippocampal Lesion
C Bilateral Cortical Control Lesion
N Normal
L Longitudinal testing
X Cross Sectional testing
XL Testing for two or three successive days

LIST OF REFERENCES

Akert, A.K., Orth, O.S., Harlow, H.F., & Schiltz, K.A. Learned behavior of rhesus monkeys following neonatal bilateral prefrontal lobotomy. Science, 1960, 132, 1944-1945.

Altman, J. Postnatal growth and differentiation of the mammalian brain with implications for a morphological theory of memory. In G.C. Quarton, T. Melnechuk, & F. O. Schmitt (Eds.), The Neurosciences. New York: Rockefeller University Press, 1967.

Altman, J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. Journal of Comparative Neurology, 1969, 137, 433-458.

Altman, J., Anderson, W.J., & Wright, K.A. Reconstitution of the external granular layer of the cerebellar cortex in infant rats after low-level X-irradiation. Anatomical Record, 1969, 163, 453-472.

Altman, J., & Das, G.D. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. Journal of Comparative Neurology, 1965, 124, 319-336.

Altman, J., Das, G.D., & Anderson, W.J. Effects of infantile handling on morphological development of the rat brain: An exploratory study. Developmental Psychobiology, 1968, 1, 10-20.

Altman, J., Wallace, R.B., Anderson, W.J., & Das, G.D. Behaviorally induced changes in length of cerebrum in rats. Developmental Psychobiology, 1968, 1, 112-117.

Angevine, J.B., Jr. Time of neuron origin in the hippocampal region, an autoradiographic study in the mouse. Experimental Neurology, 1965, Supplement 2, 1-70.

Beach, F.A. The neural basis of innate behavior: II. Relative effects of partial decortication in adulthood and infancy upon the maternal behavior of the primiparous rat. Journal of Genetic Psychology, 1938, 53, 109-148.

Benjamin, R.M., & Thompson, R.F. Differential effects of cortical lesions in infant and adult cats on roughness discrimination. Experimental Neurology, 1959, 1, 305-321.

Caley, D.W., & Maxwell, D.S. An electron microscopic study of neurons during postnatal development of the rat cerebral cortex. Journal of Comparative Neurology, 1968a, 133, 17-44.

Caley, D.W., & Maxwell, D.S. An electron microscopic study of the neuroglia during postnatal development of the rat cerebrum. Journal of Comparative Neurology, 1968b, 133, 45-70.

Caley, D.W., & Maxwell, D.S. Development of the blood vessels and extracellular spaces during postnatal maturation of rat cerebral cortex. Journal of Comparative Neurology, 1970, 138, 31-48.

Dalton, M.M., Hommes, O.R., & LeBlond, C.P. Correlation of glial proliferation with age in the mouse brain. Journal of Comparative Neurology, 1968, 134, 397-400.

DeLong, G.R. Histogenesis of fetal mouse isocortex and hippocampus in reaggregating cell cultures. Developmental Biology (in press).

Diamond, M.C., Kretch, D., and Rosenzweig, M.R. The effects of an enriched environment on the histology of the rat cerebral cortex. Journal of Comparative Neurology, 1964, 123, 111-119.

Doty, R.W. Functional significance of the topographic aspects of the retinocortical projection. In R. Lang (Ed.), The visual system: neurophysiology and psychophysics. Berlin: Springer-Verlag, 1964.

Fox, M.W., & Apelbaum, J. Ontogeny of the orienting-jump response of the rabbit. Behaviour, 1969, 35, 77-83.

Gibson, E.J., & Walk, R.D. The "visual cliff." Scientific American, 1960, 202, 64-71.

Goldman, P.S., Rosvold, H.E., & Mishkin, M. Evidence for behavioral impairment following prefrontal lobectomy in the infant monkey. Journal of Comparative and Physiological Psychology, 1970, 70, 454-463.

Guth, L., & Watson,P.K. A correlated histochemical and quantitative study on cerebral glycogen after brain injury in the rat. Experimental Neurology, 1968, 22, 590-602.

Harlow, H.F. Primary affectional patterns in primates. American Journal of Orthopsychiatry, 1960, 30, 676-684.

Harlow, H.F., Blomquist, A.J., Thompson, C.I., Schiltz, K.A., & Harlow, M. K. Effects of induction age and size of frontal lobe lesions on learning in rhesus monkeys. In R.L. Isaacson (Ed.), The neuropsychology of development. New York: Wiley, 1968.

Hays, W.L. Statistics for Psychologists. New York: Holt, Rinehart, and Winston, 1963.

Hicks, S.P. Embryogenic (developmental) disorders of the nervous system. In G.M. Shy, E.S. Goldensohn, & S.H. Appel (Eds.), The cellular and molecular basis of neurologic disease. Philadelphia: Lea and Febiger, 1968.

Hicks, S.P., & D'Amato, C.J. Radioautographic procedure. Personal communication, 1970.

Hicks, S.P., Cavanaugh, M.C., & O'Brien, E.D. Effect of anoxia on the developing cerebral cortex in the rat. American Journal of Pathology, 1962, 40, 615-635.

Hicks, S.P., & D'Amato, C.J. Cell migrations to the isocortex in the rat. Anatomical Record, 1968.

Hinde, R.A. Animal Behavior: A synthesis of ethology and comparative psychology. New York: McGraw-Hill, 1966.

Isaacson, R.L., Nonneman, A.J., & Schmaltz, L.W. Behavioral and anatomical sequelae of damage to the infant limbic system. In R.L. Isaacson (Ed.), The neuropsychology of development. New York: Wiley, 1968.

Joftes, D.L., & Warren, S. Simplified liquid emulsion radioautography. Journal of the Biological Photographic Association, 1955, 23, 145.

Kennard, M.A. Reorganization of motor function in the cerebral cortex of monkeys deprived of motor and premotor areas in infance. Journal of Neurophysiology, 1938, 1, 477-496.

Kennard, M.A. Cortical reorganization of motor function: Studies on a series of monkeys of various ages from infancy to maturity. Archives of Neurology and Psychiatry, 1942, 48, 227-240.

Kling, A., & Tucker, T.J. Sparing of function following localized brain lesions in neonatal monkeys. In R.L. Isaacson (Ed.), The neuropsychology of development. New York: Wiley, 1968.

Kosmarskaya, E.N., & Palenova, N.G. Influence of closed cranial injury on the development of the cerebral vascular system. Byulleten' Eksperimental' noi Biologii i Meditsiny, 1968, 66, 101-104

Lashley, K.S. The mechanism of vision. III. The comparative visual acuity of pigmented and albino rats. Journal of Genetic Psychology, 1930, 37, 481-484.

Lenneberg, E.H. The effect of age on the outcome of central nervous system disease in children. In R.L. Isaacson (Ed.), The neuropsychology of development. New York: Wiley, 1968.

Lore, R., & Sawatski, D. Performance of binocular and monocular infant rats on the visual cliff. Journal of Comparative and Physiological Psychology, 1969, 67, 177-181.

Malakhovskaia, D.B. Development of motor activity in rabbits during the early postnatal period. Fiziologicheskii Zhurnal SSSR, 1961, 47, 872-877.

Messier, B., & LeBlond, C.P. Preparation of coated radioautographs by dipping sections in fluid emulsion. Proceedings of the Society for Experimental Biology and Medicine, 1957, 96, 7-10.

Monnier, M., & Gangloff, H. Atlas for stereotaxic brain research in the conscious rabbit. Amsterdam: Elsevier, 1961.

Myers, R.E., Beard, R., & Adamsons, K. Brain swelling in the newborn rhesus monkey following prolonged partial asphyxia. Neurology, 1969, 19, 1012-1018.

Mykytowycz, R. Further observations on the territorial function and histology of the submandibular cutaneous (chin) glands in the rabbit, Orychtolagus cuniculus (L.). Animal Behaviour, 1965, 13, 400-412.

Nonneman, A.J., & Isaacson, R.L. Alterations in brain development resulting from hippocampal or cortical destruction in newborn and six week old kittens. Unpublished study.

Pilipenko, V.I. Development of the structural organization of the olfactory analyzer in ontogenesis. Byulleten' Eksperimental'noi Biologii i Meditsiny, 1968, 66, 105-108.

Raisler, R.L., & Harlow, H.F. Learned behavior following lesions of posterior association cortex in infant, immature, and preadolescent monkeys. Journal of Comparative and Physiological Psychology, 1965, 60, 167-174.

Raisman, G. Neuronal plasticity in the septal nuclei of the adult rat. Brain Research, 1969, 14, 25-48.

Rogers, A.W. Techniques of autoradiography. London: Elsevier, 1967.

Rose, J.E., Malis, L.E., Kruger, L., & Baker, C.P. Effects of heavy ionizing monoenergitic particles on the cerebral cortex. II. Histological appearance of nerve fibers after laminar destruction. Journal of Comparative Neurology, 1960, 115, 243-296.

Rose, J.E., & Woolsey, C.N. Organization of the mammalian thalamus and its relationships to the cerebral cortex. Electroencephalography and Clinical Neurophysiology, 1949, 1, 391-404.

Rosenblatt, J.S., Turkewitz, G., & Schneirla, T.C. Development of suckling and related behavior in neonate kittens. In E.L. Bliss (Ed.), Roots of behavior. New York: Harper and Brothers, 1962.

Ross, S., Sawin, P.B., Zarrow, M.X., & Denenberg. V.H. Maternal behavior in the rabbit. In H. Rheingold (Ed.), Maternal behavior in mammals. New York: Wiley, 1963.

Routtenberg, A., & Glickman, S.E. Visual cliff behavior in albino and hooded rats. Journal of Comparative and Physiological Psychology, 1964, 58, 140-142.

Schadé, J.P., & Baxter, C.F. Changes during growth in the volume and surface area of cortical neurons in the rabbit. Experimental Neurology, 1960, 2, 158-178.

Schadé, J.P., Van Backer, H., & Colon, E. Quantitative analysis of neuronal parameters in the maturing cerebral cortex. Progress in Brain Research, 1964, 4, 150-175.

Schiffman, H.R. Evidence for sensory dominance: Reactions to apparent depth in rabbits, cats, and rhodents. Journal of Comparative and Physiological Psychology, 1970, 71, 38-41.

Schneirla, T.C. Aspects of stimulation and organization in approach/withdrawal processes underlying vertebrate behavior development. In D.S. Lehrman, R.A. Hinde, & E. Shaw (Eds.), Advances in the study of behavior. New York: Academic Press, 1965.

Sharlock, D.P., Tucker, T.J., & Strominger, N.L. Auditory discrimination by the cat after neonatal ablation of temporal cortex. Science, 1963, 141, 1197-1198.

Shinkman, P.G. Visual depth discrimination in animals. Psychological Bulletin, 1962, 59, 489-501.

Smith, K.R., Jr. Fine structure of the central nervous system of the fetal and postnatal rabbit with special reference to the extracellular space. Anatomical Record, 1963, 145, 288.

Tigranyan, R.A. On several aspects of energy-exchange in the brain in the presence of closed intracranial injury. Ek'sperimental ev Klinikakan Bzhshkowt'yan Handes, 1968, 8, 16-20.

Tsang, Y.C. Maze learning in rats hemidecorticated in infancy. Journal of Comparative Psychology, 1937a, 24, 221-253.

Tsang, Y.C. Visual sensitivity in rats deprived of visual cortex in infancy. Journal of Comparative Psychology, 1937b, 24, 255-262.

Tucker, T.J., & Kling, A. Preservation of delayed response following combined lesions of prefrontal and posterior association cortex in infant monkeys. Experimental Neurology, 1969, 23, 491-502.

Turkewitz, G. The development of spatial orientation in relation to the effective perceptual environment in neonate rats. Dissertation Abstracts, 1967, 28, 1243B.

Van Harreveld, A., Crowell, J., & Malhotra, S.K. A study of extracellular space in central nervous system tissue by freeze-substitution. Journal of Cell Biology, 1965, 25, 117-137.

Walk, R.D. The study of visual depth and distance perception in animals. In D.S. Lehrman, R.A. Hinde, & E. Shaw (Eds.), Advances in the study of behavior. New York: Academic Press, 1965.

Walk, R.D. The development of depth perception in animals and human infants. Monographs of the Society for Research in Child Development, 1966, 31 (Whole No. 107), 82-108.

Walk, R.D., & Gibson, E.J. A comparative and analytical study of visual depth perception. Psychological Monographs, 1961, 75 (Whole No. 519).

Weller, R.O., & Wisniewski, H. Histological and ultrastructural changes with experimental hydrocephalous in adult rabbits. Brain, 1969, 92, 819-828.

Wolf, G., & DiCara, L.V. Progressive morphologic changes in electrolytic brain lesions. Experimental Neurology, 1969, 23, 529-536.

Worden, A.N., & Leahy, J.S. The behaviour of rabbits. In E.S.E. Hafex (Ed.), The behaviour of domestic animals. London: Baillière, Tindall and Cox, 1962.

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Arthur J. Nonneman was born in Chicago, Illinois on January 17, 1943. He received his elementary and secondary education in the State of Illinois public school system and was graduated from Proviso East Township High School in June, 1961. In June, 1965, he received the degree of Bachelor of Arts from Northwestern University, Evanston, Illinois. During his senior year at Northwestern he worked as a research assistant for the Association of American Medical Colleges under Dr. Edwin B. Hutchins. In September, 1965, he entered graduate school at the University of Michigan, and served as a research assistant under Dr. Robert L. Isaacson and as a teaching assistant with the Department of Psychology during the next three years. In August, 1968, he received the Master of Science degree from the University of Michigan before transferring to the University of Florida in September, 1968. At the University of Florida he continued to work as a research assistant with Dr. Isaacson while working toward the Doctor of Philosophy degree in Psychology.

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